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(57) Abstract

A mammalian high molecular weight B-cell growth factor nucleotide has been cloned and sequenced. Recombinant vectors and cells are described. Methods of providing isolated high molecular weight B-cell growth factor DNA and polypeptide sequences are disclosed, as well as methods of making transgenic animals containing or lacking the high molecular weight B-cell growth factor sequence. Clinical uses of HMW-BCGF and conjugates thereof are also described.

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HIGH MOLECULAR WEIGHT B-CELL GROWTH FACTOR: INTERLEUKIN-14

Related Applications

Field of the Invention

This invention relates to a high molecular weight B-cell growth factor (HMW-BCGF), designated Interleukin-14 (IL-14), the nucleic acid sequences encoding this HMW-BCGF, methods for cloning DNA sequences encoding HMW-BCGF protein and cell lines relating thereto, antibodies to the HMW-BCGF protein, and therapeutic uses for the nucleic acids, proteins, and antibodies disclosed herein.

Background of the Invention

Various immunoregulatory molecules are involved in the process of B-cell proliferation and differentiation. Specifically, each of the stages of B-cell proliferation and differentiation is regulated at multiple levels to insure that necessary antibody and B-cell memory can be generated to counteract any antigenic challenge without damaging the host. Disorders in any of these stages can lead to: disease in which antibody is produced, such as common variable immunodeficiency; and diseases in which abnormal accumulation of B-cells occurs, such as the various B-cell leukemias and lymphomas.

Various cytokines, including a variety of interleukins and interferons, have been described which induce B-cell proliferation. Interleukin-2, for example, is predominantly a T-cell growth factor which can induce B-cell proliferation but which cannot inhibit B-cell differentiation into Ig-secreting plasma cells. Similarly, Interleukin-4 can induce B-cell proliferation but only of activated B-cells and it cannot inhibit B-cell differentiation. Interferons are most notable for their ability to inhibit viral replication

and another cytokine, the alpha-tumor necrosis factor, is known to induce Ig secretion from B-cells.

Summary of the Invention

Isolated nucleic acid sequences which encode high molecular weight B-cell growth factors are disclosed. Based upon its isoelectric point, the human homologue of one such HMW-BCGF had been designated pI 7.8 HMW-BCGF. After further characterization, pI 7.8 HMW-BCGF also has been designated as Interleukin-14 (IL-14). A nucleic acid sequence encoding a human IL-14 is disclosed as SEQ.ID.NO.:1. The invention thus provides in a preferred embodiment isolated nucleic acid sequences corresponding to SEQ.ID.NO.:1, or variants or fragments thereof.

Substantially pure HMW-BCGF protein, and variants and fragments thereof, are also provided. The deduced amino acid sequence of a human HMW-BCGF is disclosed as SEQ.ID.NO.:2.

In another series of embodiments, cell lines and expression vectors including a nucleic acid sequence encoding a HMW-BCGF, or a variant or fragment thereof, are provided. The nucleic acid sequence may correspond to SEQ.ID.NO.:1, or to a variant or fragment thereof. Anti-sense nucleic acid sequences for the HMW-BCGF are also provided.

Methods for providing the HMW-BCGF protein of the present invention also are disclosed. For example, an expression vector including a nucleic acid sequence encoding at least a fragment of a HMW-BCGF may be introduced within a host, the host then may be allowed to express the HMW-BCGF sequences, and the protein product isolated. Preferably, the nucleic acid sequences correspond to SEQ.ID.NO.:1, or to a variant or fragment thereof.

Further methods of the invention include a method for inactivating a HMW-BCGF gene. The method includes producing a nucleic acid sequence including at least a fragment of SEQ.ID.NO.:1, introducing the sequence into a cell and

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allowing the sequence to undergo homologous recombination with the complementary, genomic DNA sequences of the host so as to inactivate the HMW-BCGF gene. Cells which have an inactivated HMW-BCGF gene may be selected for and transgenic animals may be produced.

In another embodiment of the invention, cell lines and transgenic animals with altered HMW-BCGF expression are provided. The cell lines and transgenic animals may have increased or decreased expression of HMW-BCGF or may have HMW-BCGF expression subject to altered regulatory control so as to be experimentally manipulable.

In another embodiment, antibodies, and preferably, monoclonal antibodies to a HMW-BCGF protein, or to a variant or fragment thereof, are provided.

In another embodiment of the invention, pharmaceutically effective therapeutic compositions useful for inhibiting B-cell proliferation are provided. The therapeutic composition may include a conjugate molecule such as a HMW-BCGF coupled to a toxin. The HMW-BCGF-toxin conjugate may be a fusion protein or may be produced using coupling reactions in which a HMW-BCGF is coupled to a toxin. Particularly preferred therapeutic compositions are toxins directed against cells bearing a HMW-BCGF receptor. Alternatively, the composition may be an antibody which selectively binds a HMW-BCGF so as to prevent it from interacting with its receptor and stimulating B-cell proliferation. Or, the composition may include nucleic acids which are anti-sense to, or which encode sequences which are anti-sense to, HMW-BCGF encoding sequences. Anti-sense sequences are also provided which are oligonucleotide analogues with enhanced stability and biological half-life.

In another embodiment of the invention, pharmaceutically effective therapeutic compositions useful for stimulating B-cell proliferation are provided. The therapeutic composition may include a HMW-BCGF or a variant or fragment

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thereof with B-cell proliferation stimulating activity. Alternatively, the composition may include nucleic acid sequences encoding a HMW-BCGF.

In another embodiment of the invention, a HMW-BCGF is provided which is useful in stimulating the proliferation of B-cells in vitro for cell culturing, monoclonal antibody production by B-cell hybridomas, or assays for inhibitors of B-cell proliferation or growth.

In still another embodiment of the invention, vaccines are provided which include the high molecular weight B-cell growth factors, or fragments or variants thereof, in combination with an immunogen. The vaccine further may include at least one additional immunostimulatory cytokine.

In another embodiment of the invention, a method is provided for selectively depleting a population of cells having receptors for a high molecular weight B-cell growth factor. Such cells are contacted with a cytotoxin that selectively binds to the cells. The preferred cytotoxin includes at least a fragment of a high molecular weight B-cell growth factor coupled to a toxin moiety.

In another embodiment of the invention, methods for selectively interfering with the replication of B-cells are provided. One such method involves contacting the B-cells with an anti-high molecular weight B-cell growth factor agent, such as an immunotoxin that selectively interferes with replication of B-cells expressing the high molecular weight B-cell growth factor. Other agents include high molecular weight B-cell growth factors, or fragments thereof, coupled to toxins, and antisense and vectors expressing antisense to the mRNA of a high molecular weight B-cell growth factor. The cells may be, among others, cancer cells or B-cells producing autoimmune antibodies.

In still another embodiment, methods for enhancing an immune system of a patient are provided. One method involves administering a high molecular weight B-cell growth factor to

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the patient. Another such method involves administering autologous B-cells that have been proliferated in vitro using a high molecular weight B-cell growth factor of the invention. Pharmaceutical compositions containing such autologous B-cells also are included within the scope of this invention.

In another embodiment, labeled high molecular weight B-cell growth factor is provided. Such growth factor can be used for example in vitro in assays or in vivo for imaging. Methods of imaging B-cells or other cells in vivo also are embraced by the invention. The labels and imaging techniques are otherwise conventional and are known to those of ordinary skill in the art.

Brief Description of the Drawings

Figure 1 illustrates results of a B-cell proliferation assay.

Proteins were produced as in Example 3. "Native" is native IL-14 produced from Namalva cells, "Mammalian R" is the supernatant of K562 cells transfected with SFFV.neo containing the SEQ.ID.NO.: 1 cDNA, and "Control R" is the supernatant of K562 cells transfected with SFFV.neo containing a CR2 cDNA. Data shown are the mean and SEM for triplicate wells in a representative assay.

Figure 2 illustrates results of a B-cell proliferation assay.

Proteins produced as in Example 1 were used in the Sac proliferation assay as described in Ambrus, J. et al., J. Immunol. 145: 3949 (1990). Protein obtained from the bacterial plates was diluted as indicated. "210B-I" is protein produced from the SEQ.ID.NO.:1 cDNA after IPTG induction, while "210B" protein is produced from SEQ.ID.NO.:1 cDNA without IPTG induction. "J3" is an unrelated cDNA and "J3-IPTG" if the protein produced from J3 after IPTG induction. J3-IPTG, 210B, and J3 are shown at 1:10

dilutions. "HMW-BCGF" is native IL-14 used at 10U/ml. Data shown are the mean and SEM for triplicate wells in a representative assay.

Figure 3 illustrates results of a B-cell proliferation assay.

Protein was produced from the λZAP phage (Example 3) containing SEQ.ID.NO.:1 (210B). "210B-I" is described in Figure 2 and was used at its optimal concentration for the induction of proliferation (1:10 dilution, ≃ 100 ng/ml) in the presence or absence of 10 μg/ml of the $IgG_1\kappa$ monoclonal antibodies BCGF/1/C2 ("1C2"; anti-IL-14), "BA5" (anti-IL-14 receptor), or "3A1" (recognizes a poorly characterized thymocyte antigen). Controls include the "210B" (not induced with IPTG) and a phage containing an unrelated cDNA induced with IPTG ("J3-IPTG") used at the same total protein concentration as 210B-I. Data shown are the mean and SEM for triplicate wells in a representative assay.

Figure 4 illustrates results of a B-cell proliferation assay.

Mononuclear cells were stimulated with Media alone, pokeweed mitogen "PWM" (1:100), or PWM + proteins as indicated. The same proteins were used in this figure as in Figure 1. IL-14 was used at 10U/ml while 210B-IPTG, 210B and J3-IPTG were used at a 1:10 dilution. Concentration of IgG and IgM in cell supernatants was determined by ELISA, as previously described (Ambrus, Jr., J., et al., J. Immunol. 145:3949 (1990)). Data shown are the mean and SEM for quadruplicate wells in a representative assay.

Figure 5 illustrates B-cell growth factor (HMW-BCGF; IL-14) activity of NHL-B patient cell populations.

Ion-exchange chromatographic fractions from freshly obtained NHL-B patient effusion fluids were tested for BCGF activity using a standard tritiated thymidine incorporation assay. Results from fractions of autochthonous purified

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NHL-B patient cells from two patients (105 cells/well) assayed for 72 hrs are shown.

Figure 6 illustrates the production of BCGF activity by high grade NHL-B and control cells.

Lymphoma cells obtained from effusion fluids from each of the patients described were assayed for BCGF activity after stimulation with PHA (1-4) for 48 hrs in vitro, as contrasted with fluids from patients with low grade NHL-B (a) or effusions from carcinoma patients (b, c).

Figure 7 illustrates the production of BCGF activity by NHL-B cells from patients and established cell lines.

(a) BCGF activity was assayed on anti-μ B-cells by standard tritiated thymidine incorporation assay for the four patients (1-4) and three controls (as in Figure 6).

(b) Production of BCGF activity after PHA stimulation for 48 hrs in vitro by either established NHL-B-cell lines from other NHL-B patients or the original Burkitt's Lymphoma cell line (Namalva) from which HMW-BCGF was originally described.

Detailed Description of the Invention

Definitions.

In the description that follows, a number of terms used in biochemistry, molecular biology, recombinant DNA (rDNA) technology and immunology are extensively utilized. In addition, certain new terms are introduced for greater ease of exposition and to more clearly and distinctly point out the subject matter of the invention. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Gene. A gene is a nucleic acid sequence including a promoter region operably joined to a coding sequence which may serve as a template from which an RNA molecule may be transcribed by a nucleic acid polymerase. A gene contains a promoter sequence to which the polymerase binds, an

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initiation sequence which signals the point at which transcription should begin, and a termination sequence which signals the point at which transcription should end. The gene also may contain an operator site at which a repressor may bind to block the polymerase and to prevent transcription and/or may contain ribosome binding sites, capping signals, transcription enhancers and polyadenylation signals. The promoter, initiation, termination and, when present, operator sequences, ribosome binding sites, capping signals, transcription enhancers and polyadenylation signals are collectively referred to as regulatory sequences. Regulatory sequences 5' of the transcription initiation codon are collectively referred to as the promoter region. The sequences which are transcribed into RNA are the coding sequences. The RNA may or may not code for a protein. RNA that codes for a protein is processed into messenger RNA (mRNA). Other RNA molecules may serve functions or uses without ever being translated into protein. These include ribosomal RNA (rRNA), transfer RNA (tRNA), and the anti-sense RNAs of the present invention. In eukaryotes, coding sequences between the translation start codon (ATG) and the translation stop codon (TAA, TGA, or TAG) may be of two types: exons and introns. The exons are included in processed mRNA transcripts and are generally translated into a peptide or protein. Introns are excised from the RNA as it is processed into mature mRNA and are not translated into peptide or protein. As used herein, the word gene embraces both the gene including its introns, as may be obtained from genomic DNA, and the gene with the introns excised from the DNA, as may be obtained from cDNA.

Anti-sense DNA is defined as DNA that encodes anti-sense RNA and anti-sense RNA is RNA that is complementary to or capable of selectively hybridizing to some specified RNA transcript. Thus, anti-sense RNA for a particular gene would be capable of hybridizing with that gene's RNA transcript in

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a selective manner. Finally, an anti-sense gene is defined as a segment of anti-sense DNA operably joined to regulatory sequences such that the sequences encoding the anti-sense RNA may be expressed.

cDNA. Complementary DNA or cDNA is DNA which has been produced by reverse transcription from mature mRNA. In eukaryotes, sequences in RNA corresponding to introns in a gene are excised during mRNA processing. cDNA sequences, therefore, lack the intron sequences present in the genomic DNA to which they correspond. In addition, cDNA sequences will lack the regulatory sequences which are not transcribed into RNA. To create a functional cDNA gene, therefore, the cDNA sequence must be operably joined to a promoter region such that transcription may occur.

Operably Joined. A coding sequence and a promoter region are said to be operably joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the promoter region. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of promoter function results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

If it is not desired that the coding sequence be eventually expressed as a protein or polypeptide, as in the

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case of anti-sense RNA expression, there is no need to ensure that the coding sequences and promoter region are joined without a frame-shift. Thus, a coding sequence which need not be eventually expressed as a protein or polypeptide is said to be operably joined to a promoter region if induction of promoter function results in the transcription of the RNA sequence of the coding sequences.

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene. Such transcriptional control sequences may also include enhancer sequences or upstream activator sequences, as desired.

Vector. A vector may be any of a number of nucleic acid sequences into which a desired sequence may be inserted by restriction and ligation. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include plasmids, phage, phasmids and cosmids. A cloning vector is one which is able to replicate in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a

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lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to a promoter region and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (e.g., ß-galactosidase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells or hosts.

Fragment. As used herein, the term "fragment" means both unique fragments and substantially characteristic fragments. As used herein, the term "fragment" is not to be construed according to standard dictionary definitions.

Substantially Characteristic Fragment. A "substantially characteristic fragment" of a molecule, such as a protein or nucleic acid sequence, is meant to refer to any portion of the molecule sufficiently rare or sufficiently characteristic of that molecule so as to identify it as derived from that molecule or to distinguish it from a class of unrelated molecules. A single amino acid or nucleotide, or a sequence of only two or three, cannot be a substantially characteristic fragment.

A substantially characteristic fragment of a nucleic acid sequence is one which would have utility as a probe in identifying the entire nucleic acid sequence from which it is derived from within a sample of total genomic or cDNA. Under stringent hybridization conditions, a substantially characteristic fragment will hybridize only to the sequence from which it was derived or to a small class of substantially similar related sequences such as allelic

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variants, heterospecific homologous loci, and variants with small insertions, deletions or substitutions of nucleotides or nucleotide analogues. A substantially characteristic fragment may, under lower stringency hybridization conditions, hybridize with non-allelic and non-homologous loci and be used as a probe to find such loci but will not do so at higher stringency.

A substantially characteristic fragment of a protein would have utility in generating antibodies which would distinguish the entire protein from which it is derived, an allelomorphic protein or a heterospecific homologous protein from a mixture of many unrelated proteins.

It is within the knowledge and ability of one ordinarily skilled in the art to recognize, produce and use substantially characteristic fragments of nucleic acid sequences and proteins as, for example, probes for screening DNA libraries or epitopes for generating antibodies.

Unique Fragment. As used herein, a unique fragment of a protein or nucleic acid sequence is a substantially characteristic fragment not currently known to occur elsewhere in nature (except in allelic or heterospecific homologous variants, i.e. it is present only in IL-14 or IL-14 "homologue"). A unique fragment will generally exceed 15 nucleotides or 5 amino acid residues. One of ordinary skill in the art can substantially identify unique fragments by searching available computer databases of nucleic acid and protein sequences such as Genbank (Los Alamos National Laboratories, USA), SwissProt or the National Biomedical Research Foundation database. A unique fragment is particularly useful, for example, in generating monoclonal antibodies or in screening DNA or cDNA libraries.

Stringent Hybridization Conditions. Stringent hybridization conditions is a term of art understood by those of ordinary skill in the art. For any given nucleic acid sequence, stringent hybridization conditions are those

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conditions of temperature and buffer solution which will permit hybridization of that nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions, depend upon the length of the nucleic acid sequence and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with identical sequences. Suitable ranges of such stringency conditions are described in Krause, M.H.. and S.A. Aaronson, Methods in Enzymology, 200:546-556 (1991). Stringent hybridization conditions, depending upon the length and commonality of a sequence, may include hybridization conditions of 30°C-60°C and from 5x to 0.1x SSC. Highly stringent hybridization conditions may include hybridixzation at 45°C and 0.1x SSC. Less than stringent hybridization conditions are employed to isolate nucleic acid sequences which are substantially similar, allelic or homologous to any given sequence.

Variant. A "variant" of a protein or nucleic acid or fragment thereof is meant to include a molecule substantially similar in structure to the protein or nucleic acid, or to a fragment thereof. Variants of nucleic acid sequences include sequences with conservative nucleotide substitutions, small insertions or deletions, or additions. Variants of proteins include proteins with conservative amino acid substitutions, small insertions or deletions, or additions. Thus, nucleotide substitutions which do not effect the amino acid sequence of the subsequent translation product are particularly contemplated. Similarly, substitutions of structurally similar amino acids in proteins, such as leucine for isoleucine, or insertions, deletions, and terminal

additions which do not destroy the functional utility of the protein are contemplated. Allelic variants of nucleic acid sequences and allelomorphic variants or protein or polypeptide sequences are particularly contemplated. The production of such variants is well known in the art and, therefore, such variants are intended to fall within the spirit and scope of the claims.

Substantially Similar. Two nucleic acid sequences are substantially similar if one of them or its anti-sense complement can bind to the other under strict hybridization conditions so as to distinguish that strand from all or substantially all other sequences in a cDNA or genomic library. Alternatively, one sequence is substantially similar to another if it or its anti-sense complement is useful as a probe in screening for the presence of its similar DNA or RNA sequence under strict hybridization conditions. Two proteins are substantially similar if they are encoded by substantially similar DNA or RNA sequences. In addition, even if they are not encoded by substantially similar nucleic acids, two proteins are substantially similar if they share sufficient primary, secondary and tertiary structure to perform the same biological role (structural or functional) with substantially the same efficacy or utility.

Substantially Pure. The term "substantially pure" when applied to the proteins, variants or fragments thereof of the present invention means that the proteins are essentially free of other substances to an extent practical and appropriate for their intended use. In particular, the proteins are sufficiently pure and are sufficiently free from other biological constituents of their hosts cells so as to be useful in, for example, protein sequencing, or producing pharmaceutical preparations. By techniques well known in the art, substantially pure proteins, variants or fragments thereof may be produced in light of the nucleic acid and amino acid sequences disclosed herein.

Isolated. Isolated refers to a nucleic acid sequence which has been: (i) amplified in vitro by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid sequence is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleic acid sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid sequence that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art.

Immunogenetically Effective Amount. An "immunogenetically effective amount" is that amount of an antigen (e.g. a protein, variant or a fragment thereof) necessary to induce the production of antibodies which will bind to the epitopes of the antigen. The actual quantity comprising an "immunogenetically effective amount" will vary depending upon factors such as the nature of the antigen, the organism to be immunized, and the mode of immunization. The determination of such a quantity is well within the ability of one ordinarily skilled in the art without undue experimentation.

Antigen and Antibody. The term "antigen" as used in this invention is meant to denote a substance that can induce a detectable immune response to it when introduced to an animal. Such substances include proteins and fragments thereof.

The term "epitope" is meant to refer to that portion of an antigen which can be recognized and bound by an antibody. An antigen may have one, or more than one epitope. An "antigen" is capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An "immunogen" is an antigen introduced into an animal specifically for the purpose of generating an immune response to the antigen. An antibody is said to be "capable of selectively binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The selective binding of an antigen and antibody is meant to indicate that the antigen will react, in a highly specific manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" (Ab) or "monoclonal antibody" (Mab) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of binding an antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. Single chain antibodies, humanized antibodies, and fragments thereof, also are included.

The Nucleic and Amino Acid Sequences

The invention relates to the isolation and characterization of nucleic acid sequences encoding a high molecular weight B-cell growth factors (HMW-BCGF). On the basis of its isoelectric point, the human homologue of this protein had been designated pI 7.8 HMW-BCGF. On the basis of further characterization of its properties, pI 7.8 HMW-BCGF also has been designated Interleukin-14 (IL-14). As used herein, the acronym HMW-BCGF is intended to refer not only to pI 7.8 HMW-BCGF (IL-14), but also to its allelic or

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allelomorphic variants, to its heterospecific homologues, and to variants thereof.

The nucleic acid sequence of IL-14 isolated from a human cDNA library prepared from Namalva B-cells is disclosed as SEQ.ID.NO.:1. The sequence is 1854 bp in length. There is a start codon at positions 73-75 and a stop codon (TGA) at positions 1567-1569. The open reading frame of 1497 bp encodes a protein of 498 amino acid residues. The deduced amino acid sequence corresponding to this open reading frame of SEQ.ID.NO.:1 is disclosed as SEQ.ID.NO.:2. Analysis of this sequence reveals a 15 amino acid signal peptide (residues -15 to -1) as would be expected for a secreted protein. The mature protein of 483 amino acids shows no significant overall sequence homology to any known cytokines found in the GenBank, EMBL or SwissProt databases. The deduced sequence does, however, show about 8 percent overall homology to complement Bb protein.

A recombinant IL-14 expression product from E. coli transformed with SEQ.ID.NO.:1 was subjected to cyanogen bromide digestion (Example 3). The amino acid sequences of some of the resulting fragments are disclosed as SEQ.ID.NO.:3, SEQ.ID.NO.:4 and SEQ.ID.NO.:5. It can be seen that these sequences are contained within the deduced amino acid sequence of SEQ.ID.NO.:2.

Native IL-14 from Namalva B-cells was also extracted, purified, digested and sequenced (Example 2). The amino acid sequences of some of the resulting fragments are disclosed as SEQ.ID.NO.:6, SEQ.ID.NO.:7 and SEQ.ID.NO.:8. Again, these native IL-14 fragment sequences are found to be contained within the deduced amino acid sequence of SEQ.ID.NO.:2.

Cloning and In Vitro Testing of Interleukin-14

IL-14 is a cytokine produced by malignant B-cells as well as normal and malignant T-cells. As noted above, various other cytokines, including a variety of interleukins and

interferons have been described which induce B-cell proliferation. IL-14, however, not only induces proliferation of activated B-cells but also inhibits antibody synthesis or secretion by activated B-cells. IL-14 is the only cytokine with these dual effects. Interleukin-2 is predominantly a T-cell growth factor which can induce B-cell proliferation. Unlike IL-14, it cannot inhibit B-cell differentiation into Ig-secreting plasma cells. Interleukin-4 can induce B-cell proliferation, but not of activated B-cells. Unlike IL-14, Interleukin-4 does not inhibit B-cell differentiation. Further, Interleukin-4 induces expression of CD23 on B-cells; IL-14 has no effect on CD23 expression. Interferons are most notable for their ability to inhibit viral replication; IL-14 has no effect on viral replication. Alpha-tumor necrosis factor, unlike IL-14, induces Ig secretion from B-cells. Among blood leukocytes and tumors derived from them, only B-cells have receptors for IL-14.

A cDNA encoding IL-14 polypeptide was cloned by preparing mRNA from human lymphoma cells (i.e. Namalva B-cells) that express large amounts of IL-14. The mRNA was reverse transcribed into cDNA and then ligated into a phage vector. The phage was grown on bacterial plates until plaques were visible. The plaques containing the human IL-14 cDNA were screened with anti-IL-14 monoclonal antibody BCGF/1/C2 (Ambrus, Jr., J. et al., J. Exp. Med. 162:1319 (1985) or a polyclonal rabbit antiserum to IL-14 (Goldstein, H. et al., Cell Immunol. 108:343 (1990). Positive plaques were picked, plated and re-screened. Plaques positive for both monoclonal antibody and polyclonal antiserum contained SEQ.ID.NO.:1 (Example 1).

The activity of isolated IL-14 polypeptides of the present invention was compared with the activity of native IL-14 polypeptide isolated and purified from Namalva B-cells.

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A cDNA of IL-14 (e.g. SEQ.ID.NO.:1) may be inserted into a vector, for example, a phage vector, and expressed in an appropriate host (e.g. E.coli). Protein expressed by this expression system is then separated by conventional protein separation techniques (e.g. SDS-PAGE gels). Alternately, protein expressed by an expression system may be eluted directly from agar used to grow the host (Example 3).

Native IL-14 is prepared from cells that express large quantities of the native polypeptide. Namalva cells, a human B-cell lymphoma, are particularly preferred for this purpose (Ambrus, Jr., J. and A.S. Fauci, J. Clin. Invest. 75:732 (1985)). Cell supernatants are concentrated and then separated. Fractions of the cell supernatants testing positive for a functional assay for IL-14 (see Example 4) are then separated by a conventional technique (for example fluid phase isoelectric focusing or ion exchange chromatography) and stained. Spots on the separation gel are eluted and the amino acids sequenced. In addition, proteins in gels are transblotted to polyvinylidene fluoride (PVDF) paper and peptide fragments are generated directly from the PVDF without elution and the fragments are sequenced.

Expressed IL-14 recombinant polypeptide, the native IL-14 polypeptide and peptide fragments are subjected to the following tests:

1. a test of ability of the expressed IL-14 recombinant polypeptide to induce B-cell proliferation (Example 4);

2. a test of the ability of the expressed IL-14 recombinant polypeptide to inhibit immunoglobulin secretion (Example 4);

3. a test of whether IL-14 recombinant polypeptide expressed in a vector system which fails to produce a fusion protein (e.g. SFFV. neo; Example 3) would also be recognized by antibodies against nonrecombinant IL-14 polypeptide;

4. a test of whether expressed IL-14 recombinant polypeptide produced by an expression system would induce

similar B-cell proliferation as native IL-14 polypeptide of Sac-activated B-cells (Example 4);

5. a test of whether proliferation of B-cells by expressed IL-14 recombinant polypeptide is dose dependent and/or inhibited by anti-IL-14 antibody or anti-IL-14 receptor antibodies (Example 4); and

6. a test of whether expression of IL-14 messenger RNA that hybridizes to SEQ.ID.NO.:1 is consistent with the pattern of expression of native IL-14 messenger RNA (Example 5).

Clinical Studies of Interleukin-14

The molecular basis of neoplastic B-cell growth is complex and poorly understood. Cytokines have been postulated to contribute to neoplastic cell growth, and many in vitro studies have confirmed this prediction, but little is known about the in vivo role of these growth factors. Therefore, the production of IL-14 by aggressive intermediate (large cell) or high grade non-Hodgkin's lymphomas of the B-cell type (NHL-B) in four patients with lymphomatous effusions was examined to determine appropriate conditions, targets and approaches for IL-14 based therapy.

The non-Hodgkin's lymphomas are a heterogeneous group of predominantly B-cell-derived human lymphoid malignancies (NHL-B). (Foon, K. A., and R. F. Todd. Blood 68:1-31 (1986); Warnke, R. A., et al., N. Engl. J. Med. 309:1275-1281 (1983)). Clinically, as well as biologically, the NHL-B can be categorized either as indolent, including low grade and some "intermediate" cell types in the International Working Formulation (IWF) (Cancer 49:2112-2135 (1982)), or as aggressive, including high grade and some of the kinetically active (S phase % >15) intermediate (large cell) lymphomas in the IWF. The high grade (aggressive) NHL-B are associated with prodigious growth potential, when evaluated by flow cytometry and other methods (Shackney, S. E., et al., J.

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Clin. Invest. 73:1201-1222 (1984)), often showing S phase percentages of >20 on cell cycle analysis (Bauer, K. D., et al., Cancer Res. 46:3173-3178 (1986)). Regulation of cell growth in the NHL-B remains a poorly defined area, although it has previously been demonstrated that cell growth in the NHL-B can be mediated through T cell-derived human B-cell growth factors (BCGFs) (Ford, R. J., et al., Blood 65:1335-1341 (1985)). In the high grade NHL-B, it has also been shown that the lymphoma cells secrete growth factors that stimulate in vitro cell growth in autochthonous NHL-B-cells as well as in other types of neoplastic and normal human B-cells in vitro (Sahasrabudde C. G., et al., Blood 73:1149-1155 (1989); Ford, R. J., et al., J. Exp. Med. 162:1093-1098 (1985)). Other studies have also shown that high grade Burkitt's lymphoma cell lines, such a Namalva, secrete a HMW-BCGF after stimulation with phytohemagglutinin (PHA), with a similar spectrum of growth factor activities (Ambrus, Jr., J. L., et al., J. Clin. Invest. 75:732-739 (1985); Ambrus, Jr., J. L., et al., J. Exp. Med. 162:1319-1335 (1985)). One such HMW-BCGF is IL-14, the nucleic and amino acid sequences of which are disclosed herein.

It had been hypothesized that anomalous secretion of BCGFs in neoplastically-transformed NHL-B, might establish an autocrine or paracrine mechanism by which NHL-B escape from the usual tightly-regulated control of cytokine growth factors mediating normal B-cell growth (Ford, R. J., et al., Blood 67:573-577 (1986)). While BCGF activities have been readily demonstrated in vitro, there has been little evidence supporting the importance of the BCGFs in the pathophysiology of the high grade NHL-Bs in vivo. In the following disclosure and examples, studies are described showing that IL-14 is present in the effusion fluids of NHL-B patients with high tumor cell burdens, and that freshly isolated NHL-B-cells from these effusions secrete IL-14.

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Regulation of tumor cell growth in patients with malignant lymphoma is clearly an area of central importance both biologically and therapeutically. A number of studies, particularly in aggressive intermediate (large cell) and high grade NHL-B, where cell kinetics were evaluated by flow cytometry, have shown that this parameter relates directly to patient prognosis (French, M., et al., Leuk. & Lymphoma 6:231-238 (1992); Aine, R., et al., Hematol. Oncol. 8:339-346 (1990)). Previous studies have shown that both low and high molecular weight BCGF cytokines are capable of stimulating cell proliferation in NHL-B-cells in vitro, and that cell lines from aggressive NHL-Bs secreted cytokine molecules with properties similar or identical to the IL-14 disclosed herein. (Ford, R. J., et al., Blood 65:1335-1341 (1985); Sahasrabudde C. G., et al., Blood 73:1149-1155 (1989); Aine, R., et al., Hematol. Oncol. 8:339-346 (1990)).

The results disclosed below extend the previous findings in a small but interesting group of NHL-B patients with high tumor cell burdens present in lymphomatous effusion fluids (a common finding in relapsing or widespread NHL-B) (Example 13). These NHL-B cases were quite typical of progressive or late stage aggressive intermediate and high grade lymphoma (although one patient was studied untreated at presentation), and had cytogenetic abnormalities consistent with non-random chromosomal abnormalities (Croce, C., et al., Blood 65:1-7 (1985); Yunis, J., et al., Critical Rev. Oncogen. 4:161-190 (1993)) characteristic of these lymphomas (Example 14).

Interleukin-14 is believed to be an autocrine growth factor for NHL-B cells. Cell lines were successfully established from the patients' high grade B-cell lymphomas, consistent with our previous experience that advanced stage NHL-B are more amenable to cell line establishment than earlier stage high or intermediate grade (large cell), B-cell lymphoma at initial presentation (Ford, R. J., et al. Blood 75:1311-1318 (1990)) (Example 15). This had suggested that

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an autocrine growth factor might be present in the advanced intermediate (large cell) and high grade NHL-B. The cell lines derived from these patients were similar to those previously reported, showing phenotypic concordance with the patients' freshly obtained biopsy cells, and density dependence for in vitro cell growth, again suggesting that an autocrine growth mechanism is present. In the studies disclosed herein, it is now demonstrated that the lymphoma cells from these patients express the gene for IL-14 (Examples 16, 17). They also secrete the IL-14 cytokine (Example 18) and proliferate in response to IL-14 (Example 19). This suggests that IL-14 is an autocrine growth factor for these lymphomas in vivo, although other factors could also be involved with stimulating IL-14 production in vivo. Lymphoma cells in this study required PHA stimulation for maximal IL-14 production in vitro. Since the patients' NHL-B-cells also proliferate in response to purified IL-14 in vitro, but not to a variety of other putative human B-cell stimulatory cytokines that were tested in this study, the cytokine growth factor described from these patients displays many of the characteristics of an autocrine growth factor for NHL-B (Ford R. J., et al. Curr. Top. Microbiol. Immunol. 182:341-347 (1992)).

Since exogenous IL-14 also stimulated NHL-B-cell proliferation in vitro, utilization of IL-14 is believed to be paracrine as well as autocrine. The role of PHA in up-regulating the production of HMW-BCGF, which was originally discovered serendipitously in the Namalva Burkitt's lymphoma (BL) cell lines, is of particular interest. We have shown that PHA stimulatory capacity appears to be a general property of freshly isolated, purified high grade NHL-B-cells, as well as cell lines derived from large cell and immunoblastic B-cell lymphomas, from patients with high grade B-cell lymphomas. Although PHA is generally believed to be primarily a human T cell mitogen,

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and had no direct mitogenic activity on activated normal or neoplastic human B-cells in our assays, studies using fluorescinated-PHA have shown that it binds quickly (within 5 minutes) in vitro to isolated normal and neoplastic B-cells. This indicates that this lectin can function by binding to an as yet unidentified cell surface molecule, that can trigger increased release of IL-14 from high grade NHL-B.

The demonstration of a autocrine/paracrine growth factor (AGF) for aggressive (intermediate and high grade) NHL-B suggests a variety of biological approaches to therapy for these lymphomas. This is of particular importance for patients such as those described here, as these patients were refractory to further chemotherapy or other salvage treatments, resulting in an extremely poor prognosis and consequently very short survival times. The presence of AGF-mediated tumor cell growth provides a "weak link" for therapeutic exploitation. An autocrine "loop" mechanism can be best interdicted with biological strategies through the use of biologic response modifier (BRM) agents. The isolation of a cDNA for IL-14 (HMW-BCGF) disclosed herein (Ambrus, Jr., J. L., et al., Proc. Natl. Acad. Sci. (USA) 90:6330-6334 (1993)), makes BRM approaches to therapy possible. As descriged more fully below, one may now employ antisense oligonucleotides based on the IL-14 sequence for blocking IL-14 gene expression or monoclonal antibodies blocking the IL-14 receptor for aggressive high and intermediate grade lymphoma therapy.

Preferred Embodiments

In one preferred embodiment of the present invention, nucleic acid sequences encoding a human IL-14 are isolated. The sequences may correspond exactly to SEQ.ID.NO.:1 or may represent an allelic variant found in the human population. The sequences may be isolated from a cDNA or gDNA library, or may be chemically synthesized on the basis of the sequences

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disclosed herein. In light of the present disclosure, one of ordinary skill in the art is enabled to obtain or produce isolated nucleic acid sequences corresponding to SEQ.ID.NO.:1, or to variants or fragments thereof. Particularly contemplated as falling within the spirit and scope of the claims of the present invention are variants which are naturally occurring allelic variants of the disclosed sequences and variants which are produced, by site-directed mutagenesis, chemical synthesis, or other methods which are synonymous with the disclosed sequences because of the degeneracy of the genetic code. Fragments which are particularly contemplated as falling within the spirit and scope of the claims of the present invention are unique fragments, as determined by reference to established genetic databanks, and substantially characteristic fragments which may be usefully employed as probes for the disclosed sequences or for allelic and heterospecific homologues of the disclosed sequences. In light of the present disclosure, anti-sense sequences to the disclosed sequences may be easily produced by one of ordinary skill in the art and, therefore, anti-sense sequences to the disclosed sequences, or to variants or fragments thereof, are also particularly contemplated as falling within the spirit and scope of the present invention.

In another embodiment, the present invention provides for isolating the heterospecific but homologous gene for IL-14 from any vertebrate, and in particular from non-human animals such as mice, rats, guinea pigs, hamsters, cows, pigs, sheep, horses, cats, dogs, monkeys and the like. In one approach, total mRNA can be isolated from tissues or from cell lines likely to express IL-14 (e.g. B-cells, lymph tissues or lymphomas). In general, total RNA from the selected tissue or cell culture may be isolated using any conventional methods. RNA for Northern analysis may be size-fractionated by electrophoresis or centrifugation and the RNA transcripts

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may be transferred to nitrocellulose according to conventional protocols (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Press, Plainview N.Y. (1989)).

A labeled probe (e.g. SEQ.ID.NO.:1 or a probe corresponding to a variant or fragment of SEQ.ID.NO.:1) capable of hybridizing with human IL-14 under stringent hybridization conditions can serve to identify RNA transcripts complementary to at least a fragment of the non-human IL-14 gene. For example, if Northern analysis indicates that RNA isolated from a monkey lymph tissue hybridizes with the labeled probe, then a monkey lymph cDNA library is a likely candidate for screening and identification of a clone containing the coding sequence for the monkey homologue of human IL-14.

Using Northern analysis, not only the presence but also the size of a transcript may be determined. If the length of the DNA clone identified using the probes disclosed herein is less than the length of RNA transcripts as seen by Northern analysis, this allows one to determine whether a given DNA clone is long enough to encompass the entire transcript or whether it is necessary to obtain further DNA clones. If the DNA is not long enough, it is necessary to perform several steps such as: (i) re-screening the same library with the longest probes available to identify a longer DNA; (ii) screening a different DNA library with the longest probe; or (iii) preparing a PCR primer-extended DNA library using a specific nucleotide primer corresponding to a region close to, but not at, the most 5' available region and then screening with the probe corresponding to available sequences located 5' to the primer. (See for example, Rupp et al., Neuron, 6:811-823 (1991)).

In another approach, total genomic DNA can serve as a template for PCR amplification. PCR specifically replicates a particular segment of DNA in vitro by utilizing two

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oligonucleotide primers that flank the desired DNA segment. PCR entails repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences on the DNA strands, and extension of the annealed primers with thermostable DNA polymerase. The primers are chosen to hybridize to opposite strands of the desired DNA segment and are oriented so that DNA synthesis proceeds across the segment between the two primers. Since the synthesized DNA products can also bind to the primers, multiple cycles of synthesis result in an exponential accumulation of the desired DNA segment. Mullis and Faloona, Methods Enzymol., 155:335 (1987). U.S. Patent Nos. 4,683,195 (Mullis) and 4,683,202 (Mullis).

A cDNA encoding IL-14 is cloned by determining human IL-14 nucleotide sequences that could be used as primers for polymerase chain reaction (PCR) methods. Based on these sequences, degenerate oligonucleotides are synthesized and used as primers for the polymerase chain reaction (Example 6).

Polymerase chain reaction (PCR) is run using total genomic DNA from a non-human animal as a template. Non-human genomic DNA can be obtained from many commercial sources, for example, Clontech, Palo Alto, CA (Rhesus monkey DNA: Cat. #6860-1). PCR products are size-fractionated and subcloned into plasmid vectors. The nucleotide sequence of the PCR product is then obtained using standard dideoxy sequence analysis (F. Sanger et al., 1977). To complete the sequence and establish the validity of the non-human IL-14 clone, a non-human cDNA library is screened using the PCR product(s) as probes. The probes are labeled and hybridization performed. Plaques are purified and amplified to yield high titre plate stocks. Restriction fragments are then subcloned. Sequencing is then performed using well known methods (e.g., Sanger et al., supra).

Since there is degeneracy in the genetic code, more than one codon exists for almost all the amino acids (except

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tryptophan and methionine). Moreover, the frequency of usage of any particular codon is different in non-humans as compared to humans. Taking into account codon degeneracy and human-preferred codons, oligonucleotides are synthesized by methods well known in the art. Moreover, oligonucleotides with various permutations of possible codons also may be synthesized. To avoid an excessive number, however, not all possible sequences resulting from the degeneracy of the code are synthesized. Rather, a subset of codons are chosen which have the highest frequency of occurrence.

To generate the entire coding sequence of the non-human IL-14 nucleotide, the PCR product generated from the PCR can be radioactively labeled according to the procedure of Vogelstein et al. Anal. Biochem., 132:6 (1983), and used to screen a genomic or cDNA library from cells (Example 7). From overlapping partial clones, a full-length genomic or cDNA sequence for non-human IL-14 is thus identified, and recombinant vector molecules containing the total genomic or cDNA sequences are obtained. Alternatively, targeted gene walking, as described in Example 8, may be used to generate a full length genomic or cDNA sequence for non-human IL-14.

In an alternative embodiment, antibodies reactive with human IL-14 (SEQ.ID.NO.:2) can also be raised, as described below, and used to screen an expression library for IL-14 clones. The various cDNA and/or gDNA libraries can be screened using conventional immunization techniques, such as those described in Harlowe and Lane, D., Antibodies, Cold Spring Harbor Press, Plainview, New York (1988). Antibodies prepared using purified human IL-14 fragments derived from SEQ.ID.NO.:2 as an immunogen are preferably first tested for cross reactivity with the homologue of IL-14 from other species. Other approaches to preparing antibodies for use in screening DNA libraries, as well as for use in diagnostic and research applications, are described below.

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According to the present invention, a nucleic acid sequence is "functionally equivalent" compared with SEQ ID NO: 1, if the nucleic acid sequence: (i) encodes a molecule that has the ability to bind IL-14 ligand (e.g. IL-14 receptor: Ambrus, Jr., J., et al., J. Immunol. 141:861 (1988)), but it does not necessarily bind IL-14 ligand with an affinity that is the same as that of native IL-14; and/or (ii) encodes a molecule that can: stimulate proliferation of activated B-cells and inhibit immunoglobulin secretion in tissue culture of human or other activated B-cells; or stimulate proliferation but fail to inhibit secretion; or fail to stimulate proliferation but inhibit secretion; or neither stimulate nor inhibit but can block any interaction with the ligand. Such functionally equivalent sequences are particularly contemplated to fall within the spirit and scope of the present invention.

IL-14 proteins of the invention include those containing as a primary amino acid sequence all or part of the amino acid residues substantially as depicted in SEQ.ID.NO.:2. IL-14 proteins of the invention also include variant peptide sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence, resulting in a conservative substitution.

For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include asparatic acid and glutamic acid.

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Also included within the scope of the invention are IL-14 proteins, or variants or fragments thereof, which are differentially modified during or after translation, e.g., by phosphorylation, glycosylation, crosslinking, acylation, proteolytic cleavage, or by linkage to an antibody molecule, membrane molecule or other ligand, (Ferguson et al., Ann. Rev. Biochem. 57:285-320 (1988)).

In addition, recombinant IL-14-encoding nucleic acid sequences of the invention may be engineered so as to modify processing or expression of IL-14. For example, and not by way of limitation, the IL-14 sequence may be operably joined to a promoter sequence and/or a ribosome binding site using well characterized methods, and thereby facilitate harvesting or bioavailability.

Additionally, a given recombinant IL-14 coding sequence can be mutated in vitro or in vivo, to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further in vitro modification or recombinant DNA manipulation. Any technique for mutagenesis known in the art can be used including, but not limited to, in vitro site-directed mutagenesis (Hutchinson, et al., 1978, J. Biol. Chem. 253:6551), use of TAB® linkers (Pharmacia), PCR-directed mutagenesis, and the like.

In another embodiment, the present invention also provides for the expression, isolation, and substantial purification of the IL-14 protein, or variants or fragments thereof. An IL-14 nucleotide sequence may be cloned or subcloned using any method known in the art. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, cosmids, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Viral vectors include, but are not limited to, SFFV.neo, vaccinia virus, or lambda derivatives such as, for example, λZAP phage. Plasmids

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include, but are not limited to, pBR322, pUC, or Bluescript® (Stratagene) plasmid derivatives.

Recombinant IL-14 molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc. Generally, introduction of IL-14 sequence(s) into a host is accomplished using a vector containing IL-14 DNA controlled by regulatory regions of the DNA that function in the host cell. For expression of the cDNA sequence as a translatable mRNA transcript encoding an IL-14 protein, it must be operably joined to eukaryotic or prokaryotic regulatory sequences including a promoter. Such recombinant molecules are easily prepared and identified by one of ordinary skill in the art using routine skill and without undue experimentation. A preferred embodiment of such an expression vector carrying the human IL-14 gene is described in Example 3. Cells transformed with these recombinant vectors are capable of expressing IL-14, or variants or fragments thereof. In addition, any intervening sequences present in cloned genomic sequences can be removed in vivo by culturing cells containing such vectors in appropriate eukaryotic hosts.

Once the IL-14 protein or a variant or fragment thereof is expressed, it may be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In particular, IL-14 protein may be isolated by binding to an affinity column comprising antibodies to IL-14 bound to a stationary support.

Also within the scope of the invention are nucleic acid sequences of IL-14 encoded by nucleic acid sequences derived from the same gene but lacking one or more structural features (e.g. cDNA's) as a result of alternative splicing of transcripts from a gene that also encodes the complete IL-14 gene.

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Nucleic acid sequences complementary to the DNA coding sequences of IL-14 or a variant or fragment thereof are also provided. In a particular aspect of the invention, anti-sense IL-14 oligonucleotides can be isolated or synthesized. These oligonucleotides may have activity in their own right, such as anti-sense reagents which inhibit transcription or translation, RNA function, B-cell proliferation and/or immunoglobulin secretion. Thus, the disclosed DNA sequences, or variants or fragments thereof, may be operably joined in a reversed orientation with regard to a promoter, so that a negative sense ("anti-sense") RNA results from transcription. This anti-sense RNA, when introduced into a cell, inhibits expression of the desired IL-14 product. Inhibition depends on formation of sense::anti-sense RNA duplex molecules which prevent translation of the appropriate messenger RNA and, therefore, an anti-sense transcript of sufficient length and complementarity to hybridize under cytoplasmic conditions must be chosen. In a most preferred embodiment, the anti-sense transcript is the complement of SEQ.ID.NO.:1.

In another embodiment, the present invention provides antibodies to an IL-14 protein, variant or fragment thereof. These antibodies include antibodies raised against the human IL-14 polypeptide (SEQ.ID.NO.:2) and intended to cross-react with the human and/or a non-human homologue. These antibodies are useful for diagnostic and therapeutic applications. Other antibodies include antibodies raised against variants or fragments, in particular unique fragments, of the human IL-14 sequence of SEQ.ID.NO.:2.

Antibodies raised against the human polypeptide (SEQ.ID.NO.:2), can be isolated by standard protein purification methods. Generally, a peptide immunogen is first attached to a carrier to enhance the immunogenic response. Although the peptide immunogen can correspond to any fragment of the amino acid sequence of the IL-14 protein

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or to variants of the sequence, such as the amino acid sequences corresponding to the primers and probes described, certain peptides are more likely than others to provoke an immediate response. In an alternative method of preparing antibodies reactive with IL-14, an animal is immunized with a protein expressed by a bacterial or eukaryotic cell, which protein includes at least a fragment of the human IL-14 peptide. Antibodies can also be prepared by immunizing an animal with whole cells that are expressing all or a fragment of a cDNA encoding IL-14.

In choosing a fragment of IL-14 for use as an immunogen, the amino acid sequence of IL-14 (SEQ.ID.NO.:2) may be analyzed in order to identify portions of the molecule which may be associated with increased immunogenicity. For example, the amino acid sequence may be subjected to computer analysis to identify surface epitopes which present computer-generated plots of antigenic index, an amphiphilic helix, amphiphilic sheet, hydrophilicity, and the like. Alternatively, the deduced amino acid sequences of IL-14 from different species could be compared, and relatively non-homologous regions identified. These non-homologous regions would be more likely to be immunogenic across various species.

For preparation of monoclonal antibodies directed toward IL-14, or a variant or fragment thereof, any technique which provides for the production of antibody molecules by continuous cell lines and culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (Nature, 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today, 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies, and the like, are within the ability of one of ordinary skill in the art and, therefore, within the scope of the present invention.

Further, single-chain antibody (SCA) methods are available to form anti-IL-14 antibodies (Ladner et al., U.S. Patents 4,704,694 and 4,976,778).

The monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. The present invention provides for antibody molecules as well as fragments of such antibody molecules.

In another embodiment, the present invention also provides model cell lines and animal models for assays of IL-14 function, induction, repression, activation or inhibition. In particular, using standard recombinant DNA techniques and the sequences disclosed herein, or variants or fragments thereof, the present invention provides for mammalian target cells which are engineered to express IL-14 or IL-14 anti-sense. For example, a recombinant IL-14 gene, either with endogenous or exogenous regulatory sequences, cloned according to the methods set forth above, may be inserted into cells such that IL-14 protein is expressed at much higher than normal levels and/or is expressed subject to altered regulatory control. Alternatively, an IL-14 anti-sense gene may be introduced such that IL-14 protein is expressed at much lower than normal levels and/or is expressed subject to altered regulatory control. Such model systems could be used to study the effects of IL-14 excess or depletion. The experimental model systems also may be used to study the effects of increased or decreased response to IL-14-receptor (Ambrus, Jr., J. L., et al., supra, 1988) in cell or tissue cultures, in whole animals, or in particular cells or tissues within whole animals or tissue culture systems, or over specified time intervals (e.g. embryogenesis).

In additional embodiments of the invention, a recombinant IL-14 sequence, variant or fragment thereof, may be used to inactivate the endogenous IL-14 gene by homologous

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recombination, and thereby create an IL-14 deficient cell, tissue, or animal. For example, and not by way of limitation, a recombinant IL-14 sequence may be engineered to contain an insertional mutation (e.g. the neo gene) which, when inserted into the genome of a recipient cell, tissue or animal, inactivates transcription or translation of IL-14. Such a construct may be introduced into a cell, such as an embryonic stem cell, by a technique such as transfection, transduction, microinjection, etc. (Example 9). Stem cells lacking an intact IL-14 sequence may generate transgenic animals deficient in IL-14. In a specific embodiment of the invention (Example 10), the endogenous IL-14 coding sequence of a cell may be inactivated by homologous recombination with a mutant IL-14 sequence to form a transgenic animal lacking the ability to express IL-14.

In another embodiment, a recombinant IL-14 gene is introduced into a cell line or transgenic animal in which the endogenous genomic IL-14 gene has been inactivated. The genomic IL-14 gene may be a naturally occurring null mutant or may be inactivated by homologous recombination with a recombinant construct as described above. The introduced recombinant Il-14 gene may be engineered such that it is operably joined to an exogenous promoter which is inducible or repressible by simple manipulations of experimental conditions. In this way, a cell line or transgenic animal may be created in which all IL-14 production is under experimental control.

A "transgenic animal," as used herein, is a non-human animal having cells that contain DNA which has been artificially inserted into a cell and which becomes part of the genome of the animal which develops from that cell. The preferred transgenic DNA encodes for human IL-14 protein and the IL-14 is preferably entirely foreign to the IL-14 produced by the non-human recipient animal. In a further embodiment of the invention, native IL-14 expression in an

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animal may be reduced by creating a transgenic animal expressing an amount of IL-14 anti-sense RNA effective to reduce expression of the native IL-14.

According to the present invention, IL-14 probes may be used to identify cells and tissues of animals which have the ability to transcribe and/or translate IL-14 RNA. IL-14 expression may be evidenced either by transcription of IL-14 mRNA or production of IL-14 protein. One variety of probe which may be used to detect and/or quantify IL-14 mRNA expression is a nucleic acid probe, containing a sequence corresponding to SEQ.ID.NO.:1, or a variant or fragment thereof. Detection of IL-14-encoding mRNA may be easily accomplished by any method known in the art, including, but not limited to, in situ hybridization, Northern blot analysis, or PCR related techniques. Alternatively, expression of IL-14 protein may be detected and/or quantified using the antibodies to IL-14 disclosed herein.

The above-mentioned probes may be used experimentally to identify cells or tissues which hitherto had not been shown to express IL-14. Furthermore, these methods may be used to identify the expression of IL-14 by aberrant tissues, such as malignancies.

In one series of embodiments of the present invention, methods, compositions and agents are provided which inhibit or interfere with B-cell growth or replication. These compositions include antibodies to IL-14; IL-14-toxin conjugates; variants or fragments of IL-14 capable of binding and thereby blocking IL-14 receptors without inducing B-cell proliferation; and IL-14 anti-sense nucleic acid sequences. Such compositions may be used therapeutically to treat any disease in which the cells involved express high levels of IL-14 receptor. These diseases include but are not limited to B-cell non-Hodgkin's lymphoma, B-cell acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia, B-cell lymphoma associated with chronic immunosuppression and

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Epstein-Barr virus-induced malignant transformation, systemic lupus erythematosus (See Example 11), Sjogren's syndrome, systemic necrotizing vasculitis, various forms of glomerulonephritis associated with immune complex deposition or auto-antibody deposition in the renal glomeruli, and other diseases characterized by high levels of auto-antibody production. These compositions may also be used to suppress immune responses in transplant patients to inhibit tissue rejection.

As described above, antibodies, both polyclonal and monoclonal, can be raised against the IL-14 of the invention, or against variants or fragments thereof, and then, if desired, selected on the basis of their ability to inhibit the activity of the IL-14. The term "inhibit the activity" of IL-14 means the ability to inhibit B-cell proliferation (See, e.g., the B-cell proliferative assay of Example 4), or ameliorate the negative effect of IL-14 on immunoglobulin secretion. Antibodies that "selectively bind" to the IL-14 or cells expressing the IL-14 bind only to IL-14 and not other interleukins.

IL-14, variants or fragments thereof, can also be conjugated to a toxin moiety, or expressed along with a toxin moiety as a recombinant fusion protein. The toxin moiety will bind to and enter a target cell using interaction of the IL-14 and the corresponding target cell surface receptor for IL-14. The toxic moiety to which the IL-14, variant or fragment thereof, is conjugated can be a protein such as, for example, pokeweed anti-viral protein, ricin, gelonin, abrin, diptheria exotoxin, or Pseudomonas exotoxin. The toxin moiety can also be a high energy-emitting radionuclide such as cobalt-60.

Those of ordinary skill in the art will recognize that a large variety of possible moieties can be linked to the IL-14 of the invention the recitation of examples herein is intended to be illustrative and by no means limiting. See,

for example, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J.M. Cruse and R.E. Lewis, Jr (eds). Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference.

The conjugation of IL-14 to another moiety (e.g. bacterial toxin) can be accomplished by any chemical reaction that will bind the two molecules so long as each molecule retains its respective activity. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. The preferred binding is, however, covalent binding. The covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the IL-14 of the present invention, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodimides, succinimide esters, diisocyanates, glutaraldehydes, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. (See Killen and Lindstrom, J. Immun. 133:1335-2549 (1984); Jansen, F.K., et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., supra).

Preferred linkers for coupling a moiety to IL-14, variants or fragments thereof, are described in the literature. See, for example, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing the use of MBS (M-maleimidobenzoyl-N- hydroxysuccinimide ester). See also, Umemoto et al. U.S. Patent 5,030,719, describing use of a halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. Particularly preferred linkers include: (i) EDC

(1-ethyl-3-(3-dimethylamino-propyl) carbodimide hydrochloride) (see Example 4); (ii) SMPT (4-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)-toluene) (Pierce Chem. Co., Cat. # 21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio)propionamido] hexanoate) (Pierce Chem. Co., Cat # 21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propianamide] hexanoate) (Pierce Chem. Co. Cat. # 21650G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide) (Pierce Chem. Co., Cat. # 24510) conjugated to EDC.

The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, the linker SMPT contains a sterically hindered disulfide bond, and can form conjugates with increased stability. Disulfide linkages are, in general, less stable than other linkages because the disulfide linkage is cleaved in vivo, resulting in less conjugate available. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-NHS, form esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

Fragments of the IL-14 of the present invention may also be used to inhibit B-cell proliferation by acting as competitive inhibitors of the interaction between IL-14 and the IL-14 receptor. Such fragments can easily be identified by one of ordinary skill in the art by testing such fragments for their ability to inhibit IL-14 activity on B-cells cultured in vitro. Such fragments may be synthesized using the sequences disclosed herein or, preferably, may be generated by enzymatic digestion of IL-14.

The present invention also provides for inhibition of B-cell proliferation using anti-sense nucleic acid sequences. Sequences complementary to the IL-14 sequence, or a variant or fragment thereof, may be used to inhibit the transcription and/or translation of a native IL-14 gene. Such sequences may be provided by introducing a recombinant vector including an IL-14 anti-sense gene into a host cell or host organism such that anti-sense sequences are produced within the host or, alternatively, the anti-sense may be introduced within the host as anti-sense transcript. When anti-sense transcript is employed, the sequence may be comprised either of nucleotides or nucleotide analogues with enhanced stability and biological half-life.

In another series of embodiments, the present invention provides for methods and compositions for increasing B-cell proliferation both in vitro and in vivo. Such compositions include the IL-14 of the present invention and variants and fragments thereof that induce B-cell proliferation or bind B-cell IL-14 receptors. Such compositions are useful in vitro as additives for media used in culturing B-cells, particularly for use in forming hybridomas and to increase the yield of monoclonal antibodies produced by B-cell hybridomas, and for use in assays for anti-B-cell tumor agents in which a strongly proliferating culture is desired. They also may be used to expand a population of antologous B-cells in vitro, which then are administered to a patient in need of such B-cells. They further can be labeled conventionally and used in assays to identify cell having IL-14 receptors.

Such compositions are useful in vivo whenever B-cell and, in particular, memory B-cell proliferation is desired. Thus, such compositions will have therapeutic use in vaccines in combination with immunogens to augment the memory B-cell population, to alleviate B-cell immunodeficiencies, or to stimulate B-cell replenishment after, for example, bone

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marrow donation. Vaccines has its ordinary meaning in the art. Such vaccines additionally may include other cytokines for further enhancing the immune response, such as Interleukin-4, Interleukin-2 or the CD40 ligand GP39.

The IL-14, variants or fragments thereof, of the present invention can also be administered in vivo to a subject (preferably a mammal, and most preferably a human) to expand proliferation of memory B-cells. Moreover, IL-14 can be administered in vivo to a subject to potentially increase the production of high affinity antibodies. This may seem counterintuitive at first, since one in vitro effect of IL-14 is to inhibit immunoglobulin secretion. Nevertheless, the in vivo physiologic effects of administered IL-14 occur in an environment where other naturally occurring effector mechanisms take place, and the presence of IL-14, in concert with these effectors, can lead to overall production of higher levels of high affinity antibodies.

The IL-14, variants or fragments thereof, of the present invention can also be used to augment B-cell growth and treat various primary immunodeficiencies (i.e. common variable immunodeficiency) or acquired immunodeficiencies (i.e. associated with human immunodeficiency virus infection or following radiation or chemotherapy). Furthermore, administration of IL-14, variants or fragments thereof, to subjects with various types of B-cell cancers (i.e. B-cell non-Hodgkin's lymphoma) may induce cell growth and proliferation so that these cells will become more susceptible to the effects of radiation therapy, chemotherapy, or biologic therapy.

The therapeutic compositions of the present invention may be administered in combination with other therapeutic compositions or in combination with a pharmaceutically acceptable and compatible carrier. The term "pharmaceutically acceptable and compatible carrier" as used herein, and described more fully below, means one or more

compatible solid or liquid filler diluents or encapsulating substances which are suitable for administration to a human or other animal. The term "therapeutically effective amount" is that amount of the present therapeutic compositions which produces a desired result or exerts a desired influence on the particular condition being treated. In particular, a "therapeutically effective amount" of IL-14, a variant, fragment, or conjugate thereof, or of IL-14 anti-sense transcript, ("IL-14 products") means that amount which is capable of ameliorating or delaying progression of the diseased, degenerative or damaged condition. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the symptoms to be treated and results sought. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Various concentrations may be used in preparing compositions incorporating the same active IL-14 ingredient to provide for variations in the age of the subject to be treated, the severity of the condition, the duration of the treatment and the mode of administration.

In the present invention, the term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate its application.

The term "compatible," as used herein, means that the components of the pharmaceutical compositions are capable of being commingled with the IL-14 products of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

Doses of the pharmaceutical compositions of the present invention will vary depending on the subject and upon the particular route of administration used. In general, when

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used for humans, an overall dose range of from about 1 microgram to about 300 micrograms is contemplated. Pharmaceutical compositions of the present invention can also be administered to a subject according to a variety of other, well-characterized protocols. The IL-14 products may be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof and are not excluded from the scope of this invention. Such pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicyclic, p-toluene-sulfonic, tartaric, citric, methanesulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzenesulphonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group. Thus, the present invention also provides pharmaceutical compositions, for medical use, which comprise IL-14, variants, fragments, or conjugates thereof, together with one or more pharmaceutically acceptable carriers and optionally any other therapeutic ingredients.

The compositions of the present invention include those suitable for oral, rectal, topical, nasal, ophthalmic or parenteral administration, all of which may be used as routes of administration using the IL-14 products of the present invention. Other suitable routes of administration include intrathecal administration directly into spinal fluid (CSF), direct injection into an arterial surface and intraparenchymal injection directly into targeted areas of an organ. Compositions suitable for parenteral administration are preferred. The term "parenteral" includes subcutaneous

injections, intravenous, intramuscular, and intrasternal injection or infusion techniques.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active IL-14 product into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the product into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the IL-14 product in liposomes or as a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, or an emulsion.

Preferred compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the composition, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing, wetting and/or suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, such as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectibles.

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Other delivery systems can include sustained release delivery systems. Preferred sustained release delivery systems are those which can provide for release of the IL-14 product in sustained release pellets or capsules. Many types of sustained release delivery systems are available. These include, but are not limited to: (a) erosional systems in which the IL-14 product is contained in a form within a matrix, found in U.S. Patent Nos. 4,452,775 (Kent), 4,667,014 (Nestor et al.); and 4,748,024 and 5,239,660 (Leonard) and (b) diffusional systems in which an active component permeates at a controlled rate through a polymer, found in U.S. Patent Nos. 3,832,252 (Higuchi et al.) and 3,854,480 (Zaffaroni). In addition, a pump-based hardware delivery system can be used, some of which are adapted for implantation directly into the body.

An alternative method for transporting IL-14 products is to use liposomes. Liposomes are single or multi-compartmented bodies obtained when lipids are dispersed in aqueous suspension. The walls or membranes are composed of a continuous lipid bilayer which encloses an inner aqueous space. Such vesicles can be used to encapsulate and deliver therapeutic agents. For example, International Patent No. WO 91/04014 (Collins et al.) describes a liposome delivery system in which the therapeutic agent is encapsulated within a liposome. Antibodies to IL-14 receptors, or other ligands can be added to the outer liposome layer. U.S. Patent No. 4,704,355 (Bernstein) describes methods for coupling antibodies to liposomes.

The invention will be further illustrated by the following, non-limiting examples.

EXAMPLE 1

cDNA Library Synthesis and Screening

Human B and T-cells were purified from peripheral blood as previously described (Ambrus, Jr. et al., J. Immunol.

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145:3949 (1990)). The Namalva B-cell line (Ambrus, Jr., J. and A.S. Fauci, supra, 1985)) was subcloned to identify clones producing high levels of IL-14. HL-60 cells were obtained from ATCC (Rockville, MD). U-937 cells were kindly provided by E.J. Brown (Washington University, St. Louis, MO), EL-4 cells and L3H cells by M. Thomas (Washington University, St. Louis, MO).

Messenger RNA was prepared from Namalva B-cells stimulated with phytohemagglutinin ("PHA") for 8 hours, reverse transcribed into cDNA and ligated into the ZAP vector (Stratagene, La Jolla, CA) according to standard protocols (Ausubel, F. et al., Current Protocols on Molecular Biology, J. Wiley & Sons, New York.). Phage were grown on plates containing LB agar and XL-1 Blue E. coli (Stratagene) until plaques were visible. Nylon filters soaked in IPTG were placed on the plates and the phage grown for an additional 3 hours. Filters were screened with the monoclonal antibody BCGF/1/C2 (Ambrus, Jr., J. et al., J. Exp. Med. 162:1319 (1985)) or a rabbit antiserum to IL-14 (Goldstein, H. et al., Cell. Immunol. 108:343 (1987)) according to established protocols (Ausubel, F. et al., supra)). Positive plaques were picked, replated, and re-screened four additional times. The cDNA described was positive with both BCGF/1/C2 and anti-IL-14 antiserum.

Screening of a λZAP cDNA library made from PHA stimulated Namalva cells with the monoclonal antibody BCGF/1/C2 (Ambrus, et al., supra, 1985)) and a polyclonal antisera to IL-14 resulted in the identification of a 1.8 kb cDNA clone whose nucleotide sequence is SEQ.ID.NO.: 1. The cDNA has a short 5' untranslated region followed by a start site. The coding sequence is 1494 bases and is followed by a 3' untranslated region.

The DNA sequence predicts a mature protein of 483 amino acids which is 53.1 kD (SEQ.ID.NO.: 2). Three potential N-linked glycosylation sites are predicted (amino acid

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positions 376, 387 and 390 of SEQ.ID.NO,: 2), consistent with the observation that native IL-14 is glycosylated (Ambrus, Jr., J., and A.S. Fauci, J. Clin. Invest. 75: 732 (1985)). Multiple cysteines are predicted which could potentially participate in the formation of disulfide bonds. The predicted IL-14 amino acid sequence has no significant overall sequence homology to any known cytokines. IL-14 does have 8% overall sequence homology (strongest near the N-terminus) with the complement protein Bb (Table 1), consistent with antigenic and limited functional similarities between these two proteins (Peters, M., et al., J. Exp. Med. 168: 1225 (1988), Ambrus, Jr., J., et al., J. Biol. Chem. 266: 3702 (1991)).

EXAMPLE 2

Purification and Sequencing of Native IL-14

Fifty liter batches of IL-14 were prepared from Namalva cells stimulated with 1 microgram/ml PHA in serum free media (Ambrus, Jr. and A.S. Fauci, supra, 1985)). Cell supernatants were concentrated by positive pressure filtration with membranes which exclude molecules larger than 10kD, absorbed with anti-FBS sepharose (Ambrus, Jr. et al., supra, (1985)), and then separated by hydroxyapatite chromatography (Mehta, S. et al., J. Immunol. 135: 3298 (1985)). Fractions with BCGF activity were pooled and separated by fluid phase isoelectric focusing using a Rotofor (BioRad).

Fluid phase isoelectric focusing was necessary in this procedure because IL-14 and the albumin used to grow the Namalva cells tend to form a complex which cannot be separated by standard forms of chromatography, such as ion exchange, hydrophobic interactive and reverse phase chromatography. Moreover, IL-14 and albumin are similar in molecular weights (60kD v. 67kD, respectively) so they cannot be separated by sieving chromotography and often run

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confluently even on SDS-PAGE. Isoelectric focusing can, however, separate albumin (pI 4.9) from IL-14 (pI 7.8).

Fractions with BCGF activity were pooled, separated by two-dimensional SDS-PAGE, transblotted to PVDF paper, and stained with Coomasie blue.

Two spots with molecular weights of 60 kD and isoelectric points of pI 6.7 and pI 7.8, respectively, were obtained. The cDNA disclosed as SEQ.ID.NO.:1 encodes the pI 7.8 protein.

N-terminal amino acid sequencing of the native IL-14 protein was performed by Edman degradation. In addition, cyanogen bromide digestion was performed directly on the PVDF paper and the generated fragments sequenced simultaneously by Edman degradation, such that multiple amino acids were obtained at each cycle.

The N-terminal amino acid sequence obtained from native IL-14 follows immediately after the signal peptide (positions -15 to -1) of the deduced amino acid SEQ.ID.NO.: 2 . Amino acid sequences of additional peptides obtained from native IL-14 and predicted from the IL-14 cDNA are also indicated as SEQ.ID.NO.:6 (positions 16 to 26); SEQ.ID.NO.: 7 (positions 37 to 42) and SEQ.ID.NO.: 8 (positions 179 to 188).

EXAMPLE 3

Expression of Cloned IL-14

A. Production of cloned HMW-BCGF using ZAP phage: Lambda-ZAP phage (Stratagene) containing the IL-14 of SEQ.ID.NO.: 1 or control cDNAs were grown in top agar containing XL-1 Blue *E. coli* with or without 0.4 mM IPTG for 12 hours at 37°C. Proteins were then eluted from the top agar with PBS containing 0.1% CHAPS and 1 mM PMSF. Proteins for use in functional assays (Example 4) were dialyzed against RPMI and absorbed with De-Toxigel (Pierce Chemical Co.).

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B. Western Blot Assay: The assay has been previously described (Ambrus, Jr. J., et al., (1985)). Briefly, expressed proteins were size fractionated in 10% SDS-PAGE gels and transblotted to Immobilon-P membranes (Millipore, Bedford, MA). Filters were blocked in 1% casein and then incubated sequentially with anti-IL-14 antibodies and ^{125}I-Protein G (Dupont/New England Nuclear). IL-14 containing bands were identified by autoradiography.

Expression of recombinant IL-14 using the λZAP phage resulted in a 60 kD ß-galactosidase fusion protein ("210B"). To prove that the band designated "210B protein" was recombinant, IL-14 was subjected to amino acid sequencing by Edman degradation after digestion with cyanogen bromide. Peptides sequenced from recombinant 210B protein are SEQ.ID.NOS.: 3, 4 and 5 and are included within the deduced amino acid sequence of IL-14 (SEQ.ID.NO.: 2). Furthermore, cloned 210B protein was recognized by anti-IL-14 antibodies in Western blot assays.

C. Production of HMW-BCGF using SFFV.neo: The previously published protocol for expression of CR2 in K562 cells using SFFV.neo was utilized (Carel, J. et al., supra). In brief, K562 cells were electroporated with fusion plasmid and then selected in media containing G418. Supernatants containing IL-14 or control proteins were harvested and used directly in assays.

Expression of recombinant IL-14 in K562 cells using the expression plasmid SFFV.neo (Carel, supra) tested whether bacterial proteins, or abnormal protein folding generated by bacteria, might be contributing to any differences between native and recombinant IL-14. Recombinant IL-14, in the absence of any fusion protein, was secreted into the supernatant of K562 cells transfected with the fusion plasmid. Western blot analysis demonstrated that the recombinant IL-14 produced in this way was similar in molecular weight to native IL-14 and recognized by IL-14

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antibodies. Figure 1 demonstrates that the recombinant IL-14 produced by the SFFV.neo expression system induced ^{3}H-thymidine incorporation by Sac-activated B-cells equivalent to induction of ^{3}H-thymidine incorporation by optimal doses of native IL-14 (See test described in Example 4). Unfortunately, our transfections using SFFV.neo and K562 cells have all been transient.

EXAMPLE 4

B-Cell Functional Assays

B-cell proliferation was determined by ^{3}H-thymidine incorporation of B-cells which had been activated with a 1:25,000 dilution of Staphyloccocus aureus Cowen I ("Sac"; Immunoprecipitin, Bethesda Research Laboratories, Gaithersburg, MD) for 72 hours and then cultured with cytokines for an additional 72 hours, as previously described (Ambrus, Jr., J. et al., J. Immunol. 145: 1990, supra)). Immunoglobulin secretion by mononuclear cells cultured in the presence and absence of pokeweed mitogen (1:100;BRL) and cytokines for 10 days was determined by ELISA, as previously described (Ambrus, Jr., J. et al., id.)).

Native IL-14 was defined on the basis of its ability to induce proliferation of activated B-cells. IL-14 is distinguished from other BCGFs by its ability to inhibit immunoglobulin secretion (Ambrus, Jr., J. et al., id)). We therefore examined the ability of recombinant IL-14 to induce B-cell proliferation and inhibit immunoglobulin secretion. Figure 2 demonstrates that the recombinant IL-14 produced from λZAP induced five-fold enhancement in ^{3}H-thymidine incorporation of Sac-activated B-cells above the background produced by Sac-activation alone. The increase in ^{3}H-thymidine incorporation induced by recombinant IL-14 was dose dependent, and specifically inhibited by anti-IL-14 or anti-IL-14 receptor antibodies (1C2, BA5, respectively, in Figure 3). However, this recombinant IL-14 did not induce as much ^{3}H-thymidine incorporation as native IL-14 (Figure 3).

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Recombinant IL-14 inhibited immunoglobulin secretion by PWM-stimulated mononuclear cells. Figure 4 demonstrates that the recombinant IL-14 produced with λZAP inhibited immunoglobulin secretion as well as native IL-14. Thus, recombinant IL-14 induced the same activities as native IL-14, although induction of proliferation was more sensitive than inhibition of immunoglobulin secretion to the differences in the molecule and/or the contaminating proteins present when it is made in bacteria.

EXAMPLE 5

Messenger RNA Expression

Namalva B-cells and T-cells were lysed with guanidine isothiocyanate and total cytoplasmic RNA isolated by centrifugation over cesium chloride cushions (Goldstein, H. et al., supra). RNA was then size-fractionated by electrophoresis in 1% agarose-formaldehyde gels, transferred to GeneScreen hybridization membranes (Dupont/New England Nuclear), and hybridized to a ^{32}P-labeled cDNA probe. A beta-actin probe was kindly provided by M. Thomas (Washington University). After hybridization, the filters were washed and exposed to Kodak XAR-5 film for 4-24 hours.

Previous experiments performed with monoclonal antibodies to IL-14 demonstrated that production of IL-14 by Namalva cells is increased after stimulation with PHA (Ambrus, Jr., J. and A.S. Fauci, supra). Furthermore, IL-14 production by normal T-cells was identified 8-12 hours after PHA stimulation, and generally absent 36-48 hours after PHA stimulation (Ambrus, Jr., J., et al., in Mechanisms of Lymphocyte Activation and Immune Regulation, pp. 163-175, (eds. S. Gupta et al.), Plenum Publishing Corp., San Fransisco, 1987).

We used a cDNA probe derived from the IL-14 cDNA of SEQ.ID.NO.: 1 to determine whether the expression of IL-14 mRNA is consistent with the production of IL-14 noted

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previously. Northern blot analysis demonstrated that the mRNA which hybridizes to the IL-14 cDNA is upregulated in Namalva cells after 8 hours of PHA stimulation. Furthermore, such analysis demonstrated that IL-14 mRNA is expressed 8-12 hours after PHA stimulation and gone by 48 hours after PHA stimulation. The T-cells of some donors expressed more IL-14 mRNA at 8 hours after PHA stimulation than the T-cells of the donor shown, but all ceased to express IL-14 mRNA by 36-48 hours after PHA stimulation. Thus, the mRNA expression of IL-14 is consistent with the previous data on IL-14 protein production (Ambrus, Jr., J. and A.S. Fauci, supra).

EXAMPLE 6

PCR Amplification of Mammalian Genomic DNA

A portion of the human IL-14 gene is amplified from human Namalva cell DNA using the polymerase chain reaction technique (Saiki, R.K., et al., 1985, Science 230 1350-1354). A 100 µl reaction contains 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.001% (w/v) Gelatin, 2mM MgCl2, 200 µM dNTPs, 1.5 µM sense primer, 1.5 µM antisense primer, 2.5 units Taq Polymerase (Perkin Elmer Cetus), and 1.0 µg of human Namalva cell DNA. The DNA Thermal-cycler (Perkin Elmer Cetus, Model N801) is programmed for the following incubations:

1. 94°C, 2 min. (initial denaturation)
2. 94°C, 1 min. (denaturation)
3. 50°C, 1 min. (annealing)
4. 72°C, 3 min. (elongation)
5. Steps 2-4 cycle 50 times (amplification)
6. 4°C, Soak (storage)

The DNA amplified in this reaction is electrophoresed on 5% polyacrylamide gels to verify band length. If the size is determined to be correct, the DNA is purified by phenol extraction, then digested with EcoRI and BamHI to remove the termini. The DNA is then ligated into the EcoRI/BamHI site

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of vector pUC19 (New England Biolabs). The DNA is transformed into E. coli strain DH5-alpha made competent by the $CaCl_2$ procedure (Hanahan, D., 1983, J. Mol. Biol. 155:557). The human IL-14 is then sequenced by the chain-termination method (Sanger, F. et al., 1977, Proc. Natl. Acad. Sci. USA, 74:5463).

Monkey IL-14 DNA can be amplified from 1.0 µg of total genomic Rhesus DNA (Clontech #6860-1) using the exact procedure described above for human DNA.

EXAMPLE 7

Cloning Procedure for Non-human IL-14

The oligonucleotide generated from the polymerase chain reactions described in Example 6, is radioactively labeled according to the procedure described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Plainview NY (1989). These oligonucleotides are used to screen a genomic library from a non-human cell line. Alternatively, a λgt11 cDNA library prepared from mRNA from a non-human (e.g., monkey) cell line is used. Construction of these libraries follows the procedure of Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Plainview NY (1989). Alternatively, a commercially available genomic DNA library, available from Clontech (Palo Alto, CA), can be used. Hybridization conditions are as described by Cate et al., Cell, 45:165 (1986), except that the final wash in tetramethyl ammonium chloride is omitted. DNA inserts from positive plaques are subcloned directly into the plasmid vector pBluescript Sk+ (Stratagene, Inc. San Diego, CA). Positive plasmid subclones are identified by colony hybridization, with the use of the same oligonucleotide hybridization probe. Minipreparations of plasmid DNA are prepared from positive colonies.

The nucleotide sequence immediately upstream from the oligonucleotide binding site is determined by double strand

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sequencing (Chen and Seeburg, DNA, 4:165 1985), using ^{32}P end-labeled oligonucleotide as sequencing primer and non-radioactive nucleotides in the extension reactions. Subclones whose codon order upstream from the priming site match the known human IL-14 amino acid sequence are sequenced in their entirety by the dideoxy chain termination method, with either the Klenow fragment of Escherichia coli DNA polymerase I or modified bacteriophage T7 DNA polymerase (Sequence; United States Biochemicals) in the extension reactions. Subclones are sequenced beginning at their termini, in both directions from a set of restriction sites. Clones are obtained whose codon order is at least partially similar to the amino acid sequence of human IL-14. A full-length genomic or cDNA sequence for non-human IL-14 is assembled from overlapping partial clones.

EXAMPLE 8

Targeted Gene Walking

Targeted gene walking (TGW) is a modification of a standard polymerase chain reaction (PCR) that allows amplification of unknown DNA sequences adjacent to short segments of known sequence. (Parker et al., Nucl. Acids Res., 19:3055, (1991)). Unlike conventional PCR techniques that amplify DNA sequences between two known primer sites, TGW can amplify DNA adjacent to one such site. Thus, TGW can serve as a replacement for conventional cloning and library screening methods for isolating sequences upstream or downstream from known sequences. The procedure can be used to isolate genes from any starting DNA template for which a limited amount of sequence information is known.

First, several standard PCR reactions are run in parallel using one "targeted primer" and different "walking primers." The targeted primer is a sequence-specific primer exactly complementary to a known sequence on the DNA molecule of interest, and is directed towards unknown adjacent

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sequences. The walking primers are non-specific sequences not complementary to DNA near the target primer. The walking primers can be any oligonucleotides unrelated to the target primer sequence. In the first series of PCRs, products are produced only when a walking primer anneals to a DNA strand contiguous with and complementary to the strand to which the targeted primer has hybridized. The PCR products of interest are preferably within the 5 kilobase size range. Amplification products are produced with as many as 60% mismatched nucleotides within the walking primer relative to DNA template. Perfect base-pairing is required only for the first two 3' nucleotides of the walking primer, but partial homology is tolerated otherwise. Annealing temperature is a key variable in determining the number of PCR products, as identified by agarose gel electrophoresis.

Second, an oligomer extension assay is performed using an "internal detection primer." This primer represents known sequences between the previous two primers, contiguous with the targeted primer. The internal detection primer is kinased with ^{32}P-λATP, then used in a single PCR cycle with DNA from the first PCR as template. This extension identifies products in the first PCR contiguous with the targeted primer. These new products are identified by agarose gel electrophoresis and autoradiography. Any products that do no hybridize to the internal detection primer represent non-contiguous amplification products produced by any subset of the primers.

Last, bands identified in the oligomer extension assay are excised from the gel, and reamplified by standard PCR using target primer and the walking primer that produced the band initially. This new PCR band is then sequenced directly to provide previously unknown sequence information.

To extend information in the opposite direction, complements are made of the targeted and internal detection primers, and their order is reversed in the protocol.

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EXAMPLE 9

Construction of Transgenic Animals

Methods for purification of DNA for microinjection are well known to those of ordinary skill in the art. See, for example, Hogan et al., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1986); and Palmer et al., Nature, 300: 611 (1982).

A variety of methods are available for the production of transgenic animals associated with this invention. DNA can be injected into the pronucleus of a fertilized egg before fusion of the male and female pronuclei, or injected into the nucleus of an embryonic cell (e.g., the nucleus of a two-cell embryo) following the initiation of cell division (Brinster et al., Proc. Nat. Acad. Sci. (USA), 82: 4438-4442 (1985)). Embryos can be infected with viruses, especially retroviruses, modified to bear IL-14 nucleotide sequences of the invention.

Pluripotent stem cells derived from the inner cell mass of the embryo and stabilized in culture can be manipulated in culture to incorporate IL-14 sequences of the invention. A transgenic animal can be produced from such cells through implantation into a blastocyst that is implanted into a foster mother and allowed to come to term.

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY), Harlan Sprague Dawley (Indianapolis, IN), etc. Swiss Webster female mice are preferred for embryo retrieval and transfer. $B6D2F_1$ males can be used for mating and vasectomized Swiss Webster studs can be used to stimulate pseudopregnancy. Vasectomized mice and rats can be obtained from the supplier.

The procedures for manipulation of the rodent embryo and for microinjection of DNA into the pronucleus of the zygote are well known to those of ordinary skill in the art (Hogan et al., supra). Microinjection procedures for fish,

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amphibian eggs and birds are detailed in Houdebine and Chourrout, Experientia, 47: 897-905 (1991). Other procedures for introduction of DNA into tissues of animals are described in U.S. Patent No. 4,945,050 (Sandford et al., July 30, 1990).

Female mice six weeks of age are induced to super-ovulate with a 5 IU injection (0.1 cc, ip) of pregnant mare serum gonadotropin (PMSG; Sigma) followed 48 hours later by a 5 IU injection (0.1 cc, ip) of human chorionic gonadotropin (hCG; Sigma). Females are placed with males immediately after hCG injection. Twenty-one hours after hCG, the mated females are sacrificed by CO_2 asphyxiation or cervical dislocation and embryos are recovered from excised oviducts and placed in Dulbecco's phosphate buffered saline (DPSS) with 0.5% bovine serum albumin (BSA; Sigma). Surrounding cumulus cells are removed with hyaluronidase (1 mg/ml). Pronuclear embryos are then washed and placed in Earle's balanced salt solution containing 0.5% BSA (EBSS) in a 37.5°C incubator with a humidified atmosphere at 5% CO_2, 95% air until the time of injection.

Randomly cycling adult female mice are paired with vasectomized males. Swiss Webster or other comparable strains can be used for this purpose. Recipient females are mated at the same time as donor females. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5% avertin per gram of body weight. The oviducts are exposed by a single midline dorsal incision. An incision is then made through the body wall directly over the oviduct. The ovarian bursa is then torn with watchmakers forceps. Embryos to be transferred are placed in DPBS and in the tip of a transfer pipet (about 10-12 embryos). The pipet tip is inserted into the infundibulum and the embryos transferred. After the transfer, the incision is closed by two sutures.

The procedure for generating transgenic rats is similar to that of mice See Hammer et al., Cell, 63:1099-1112

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(1990). Thirty day-old female rats are given a subcutaneous injection of 20 IU of PMSG (0.1 cc) and 48 hours later each female is placed with a proven male. At the same time, 40-80 day old females are placed in cages with vasectomized males. These will provide the foster mothers for embryo transfer. The next morning females are checked for vaginal plugs. Females who have mated with vasectomized males are held aside until the time of transfer. Donor females that have mated are sacrificed (CO_2 asphyxiation) and their oviducts removed, placed in DPSS with 0.5% BSA and the embryos collected. Cumulus cells surrounding the embryos are removed with hyaluronidase (1 mg/ml). The embryos are then washed and placed in EBSS (Earle's balanced salt solution) containing 0.5% BSA in a 37.5°C incubator until the time of microinjection.

Once the embryos are injected, the live embryos are moved to DPBS for transfer into foster mothers. The foster mothers are anesthetized with ketamine (40 mg/kg, ip) and xylazine (5 mg/kg, ip). A dorsal midline incision is made through the skin and the ovary and oviduct are exposed by an incision through the muscle layer directly over the ovary. The ovarian bursa is torn, the embryos are picked up into the transfer pipet, and the tip of the transfer pipet is inserted into the infundibulum. Approximately 10-12 embryos are transferred into each rat oviduct through the infundibulum. The incision is then closed with sutures, and the foster mothers are housed singly.

Methods for the culturing of ES cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation; and direct injection are well known to those of ordinary skill in the art. See, for example, Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E.J. Robertson, ed., IRL Press (1987). Selection of the desired clone of IL-14-containing ES cells

is accomplished through one of several means. Although embryonic stem cells are currently available for mice only, it is expected that similar methods and procedures as described and cited here will be effective for embryonic stem cells from different species as they become available.

In cases involving random gene integration, a clone containing the IL-14 sequence(s) of the invention is co-transfected with a gene encoding neomycin resistance. Alternatively, the gene encoding neomycin resistance is physically linked to the IL-14 sequence. Transfection is carried out by any one of several methods well known to those of ordinary skill in the art (E.J. Robertson, supra). Calcium phosphate/DNA precipitation, direct injection, and electroporation are the preferred methods. Following DNA introduction, cells are fed with selection medium containing 10% fetal bovine serum in DMEM supplemented with G418 (between 200 and 500 μg/ml biological weight). Colonies of cells resistant to G418 are isolated using cloning rings and expanded. DNA is extracted from drug resistant clones and Southern blotting experiments using a transgene-specific DNA probe are used to identify those clones carrying the IL-14 sequences. In some experiments, PCR methods are used to identify the clones of interest.

DNA molecules introduced into ES cells can also be integrated into the chromosome through the process of homologous recombination . Copecchi, Science, 244: 1288-1292 (1989). Direct injection results in a high efficiency of integration. Desired clones are identified through PCR of DNA prepared from pools of injected ES cells. Positive cells within the pools are identified by PCR subsequent to cell cloning. DNA introduction by electroporation is less efficient and requires a selection step. Methods for positive selection of the recombination event (e.g., neo resistance) and dual positive-negative selection (e.g., neo resistance and gancyclovir resistance) and the subsequent

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identification of the desired clones by PCR have been described by Copecchi, supra and Joyner et al., Nature, 338: 153-156 (1989), the teachings of which are incorporated herein by reference.

Naturally cycling or super-ovulated female mice mated with males are used to harvest embryos for the implantation of ES cells. It is desirable to use the C57B strain for this purpose when using mice. Embryos of the appropriate age are recovered approximately 3.5 days after successful mating. Mated females are sacrificed by CO_2 asphyxiation or cervical dislocation and embryos are flushed from excised uterine horns and placed in Dulbecco's modified essential medium plus 10% calf serum for injection with ES cells. Approximately 10-20 ES cells are injected into blastocysts using a glass microneedle with an internal diameter of approximately 20 μm.

Randomly cycling adult female mice are paired with vasectomized males. Mouse strains such as Swiss Webster, ICR or others can be used for this purpose. Recipient females are mated such that they will be at 2.5 to 3.5 days post-mating when required for implantation with blastocysts containing ES cells. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5% avertin per gram of body weight. The ovaries are exposed by making an incision in the body wall directly over the oviduct and the ovary and uterus are externalized. A hole is made in the uterine horn with a 25 gauge needle through which the blastocysts are transferred. After the transfer, the ovary and uterus are pushed back into the body and the incision is closed by two sutures. This procedure is repeated on the opposite side if additional transfers are to be made.

Tail samples (1-2 cm) are removed from three week old animals. DNA is prepared and analyzed by Southern blot or PCR to detect transgenic founder (F_0) animals and their

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progeny (F_1 and F_2). In this way, animals that have become transgenic for the desired IL-14 sequences are identified. Because not every transgenic animal expresses IL-14, and not all of those that do will have the expression pattern anticipated by the experimenter, it is necessary to characterize each line of transgenic animals with regard to expression of the IL-14 in different tissues.

Procedures for the production of non-rodent mammals and other animals have been discussed by others. See Houdebine and Chourrout, supra; Pursel et al., Science 244: 1281-1288 (1989); and Simms et al., Bio/Technology, 6: 179-183 (1988).

EXAMPLE 10

Protocol for Inactivating HMW-BCGF in a Mouse

Mouse genomic clones are isolated by screening a human genomic library with an IL-14 probe (SEQ.ID.NO.: 1). Duplicate lifts are hybridized with a radiolabeled probe by established protocols (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y. (1989)). Plaques that correspond to positive signals on both lifts are isolated and purified by successive screening rounds at decreasing plaque density. The validity of the isolated clones is confirmed by nucleotide sequencing.

The genomic clones are used to prepare a gene targeting vector for the deletion of IL-14 in mouse embryonic stem cells by homologous recombination. A neomycin resistance gene (neo) with its transcriptional and translational signals, is cloned into convenient sites that are near the 5' end of the IL-14 gene. This will disrupt the coding sequence of IL-14 and allow for selection by the drug Geneticin (G418) by embryonic stem (ES) cells transfected with the vector. The Herpes simplex virus thymidine kinase (HSV-tk) gene is placed at the other end of the genomic DNA as a second selectable marker. Only stem cells with the neo gene will grow in the presence of this drug.

Random integration of this construct into the ES genome will occur via sequences at the ends of the construct. In these cell lines, the HSV-tk gene will be functional and the drug gancyclovir will therefore be cytotoxic to cells having an integrated sequence of the mutated IL-14 coding sequence.

Homologous recombination will also take place between homologous DNA sequences of the ES IL-14 sequence and the targeting vector. This usually results in the excision of the HSV-tk gene because it is not homologous with the IL-14 sequence.

Thus, by growing the transfected ES cells in G417 and gancyclovir, the cell lines in which homologous recombination has occurred will be highly enriched. These cells will contain a disrupted coding sequence of IL-14. Individual clones are isolated and grown up to produce enough cells for frozen stocks and for preparation of DNA. Clones in which the IL-14 sequence has been successfully targeted are identified by Southern blot analysis. The final phase of the procedure is to inject targeted ES cells into blastocysts and to transfer the blastocysts into pseudopregnant females. The resulting chimeric animals are bred and the offspring are analyzed by Southern blotting to identify individuals that carry the mutated form of the gene in the germ line. These animals will be mated to determine the effect of IL-14 deficiency on murine development and physiology.

EXAMPLE 11

HMW-BCGF Receptors in B-cells of Subjects with SLE

The presence of IL-14 receptors in B-cells from patients with systemic lupus erythematosus (SLE) was evaluated. Freshly isolated B-cells from eleven patients with SLE were studied for their expression of IL-14 receptors. All patients were found to have greater numbers of IL-14 receptor expressing B-cells (65-85% positive using the monoclonal antibody BA5) than normal, unstimulated B-cells (1-10%

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positive). However, IL-14 receptor expression did not increase in the SLE B-cell population after in vitro Sac activation. After Sac activation, 15-40% of normal B-cells expressed IL-14 receptors. The increased numbers of IL-14 receptor-expressing B-cells in the peripheral blood of SLE patients may be due to both subpopulation selection and in vivo activation.

Additional experiments were performed using B-cells from seven untreated SLE patients with inactive disease and seven normal controls to examine several different markers purported to identify different B-cell subpopulations. B-cells were purified from peripheral blood and analyzed for expression of a variety of cell surface antigens by flow cytometry using fluorescent labeled monoclonal antibodies. The SLE patients had statistically significantly more BA5 (IL-14 receptor) positive B-cells in their peripheral blood than normal controls. Moreover, the SLE patients had a tendency towards having more CD5-positive B-cells than normal controls. The other B-cell surface antigens (CD21, CD38, and CD73) were expressed equally between SLE patients and normal patients. Thus, SLE patients may have normal representation of certain, but not all, B-cell populations. The increased expression of HMW-BCGF receptors on B-cells of subjects with SLE, a disease characterized by abnormal B-cell hyperactivity and antibody production, suggests that IL-14 toxin conjugates could eliminate abnormal B-cells involved in pathogenesis of this disease.

EXAMPLE 12

Conjugation of HMW-BCGF to Pokeweed Anti-viral Protein

A method adapted from D.E. Myers et al., J. Immunol. Methods 136:221-238, (1991) is utilized. In brief, isolated and purified recombinant IL-14 of the invention is placed in phosphate-buffered saline, pH 7.5 at 10 mg/ml and reacted with a 3:1 molar excess of SPDP (N-succinimidyl

3-(2-pyridyldithio) propionate). SPDP is dissolved in dimethyl sulfoxide (DMSO) at a concentration of 64mM and diluted 1:10 in PBS just before use. Purified pokeweed anti-viral protein (PAP) is dissolved in PBS at 10mg/ml and reacted with a 3-fold molar excess of 2-iminothiolane HCl (prepared as a 20mM solution in 50mM sodium phosphate, pH 8.6). Conjugated proteins are purified by gel filtration and reacted with each other for 2 hr at room temperature at a final molar ratio of 3.5:1 PAP to IL-14. Conjugated proteins are then purified by gel filtration.

EXAMPLE 13

Patient population.

For these studies, we utilized patients that either presented untreated with high tumor burden lymphomatous effusions (1 patient), or who had relapsed after achieving a partial or complete remission in response to frontline therapy (3 patients) for aggressive (large cell) NHL-B (e.g. CHOP-Bleo, etc.). In two of the cases, the patients had also failed salvage chemotherapy after relapse, and were considered refractory to further chemotherapy. All of these patients had lymphoma cell counts of $> 3 \times 10^6$ cells/ml in their effusion fluids. In all cases, pleural effusion or ascitic fluids were removed by standard paracentesis procedures and collected in sterile 1 liter bottles. The effusions were routinely received in the laboratory and prepared for study within one hour of removal from the patient. The tumor cells were initially pelleted from the effusion fluids, with the fluid and cellular components separately analyzed.

Microscopically, all cases were diagnosed as consistent with large cell, Non-Hodgkin's lymphoma on H&E stained cytospin preparations. Immunophenotyping by flow cytometry, utilizing a standard battery of monoclonal antibodies, confirmed that the lymphoma cells were in each case high

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grade, large cell NHL-B. Controls consisted of effusions from patients with low grade NHL-B or from patients with non-hematologic neoplasms (e.g. breast carcinoma). Cytogenetic analysis of NHL-B-cells from the effusions showed aneuploid karyotypes of the hyperdiploid or hypertetraploid type, with abnormalities present in chromosome 14 of the non-random type (t(14;18)) or indeterminate type in 3/4 patients and deletions in chromosome 6q in 2/4. Multiple random structural abnormalities were also observed in each of the cases. Cell lines were established from the NHL-B-cells recovered from the effusion patients in each of the four patients, that had identical immunophenotypic and cytogenetic profiles to the originally obtained lymphoma cell specimens.

EXAMPLE 14

Lymphoma cell characterizations.

Therapeutic or diagnostic thoracentesis or paracentesis was aseptically performed on patients with lymphomatous effusions, using standard protocols. Freshly obtained effusion fluid was centrifuged to remove lymphoma cells. The effusion fluids were immediately treated with 0.1 mM PMSF (Sigma, St. Louis) to retard proteolysis and the cellular and fluid components of the specimens were processed. The lymphoma cells were immunophenotyped by staining with a standard panel of monoclonal antibodies (Becton-Dickinson, San Jose, CA) to T and B-cell antigens (CD2, 4, 8, 10, 19, 20, 22, sIg, k, λ, etc.) and analyzed by flow cytometry (Ford, R. J., et al. J. Exp. Med. 162:1093-1098 (1985); Aine, R., et al. Hematol. Oncol. 8:339-346 (1990)). The lymphoma cells were purified by E-rosetting with SRBC and then depleted of residual contaminating inflammatory or mesothelial cells, with combinations of MAb with magnetic beads attached to anti-mouse Igs. (Sahasrabudde C. G., et al. Blood 73:1149-1155 (1989); Funderud, S., et al. Eur. J.

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Immunol. 20:201-207 (1991)). Final lymphoma cell populations contained >98% monotypic lymphoid tumor cells with a viability of >90% by trypan blue analysis.

Cytogenetic analyses were carried out as previously described. (Keating, M. L., et al. Leukemia Res. 11:119-133 (1987); Ford, R. J., et al. Blood 75:1311-1318 (1990)). Karyotyping was performed by examining at least twelve metaphases from G-banded slide preparations. After photographing the G-banded metaphases, the chromosomes were cut out and arranged for karyotyping according to ISCN formulas as previously described (Ford, R. J., et al. Blood 75:1311-1318 (1990)).

EXAMPLE 15

Establishment of lymphoma cell lines.

Freshly obtained, fully characterized lymphoma cells were cultured at varying concentrations in RPMI 1640 and 20% fetal calf serum (FCS; Hyclone, Ogden, UT.) at 37°C in a 5% CO_2 incubator. Cell lines were established as previously described (Ford, R. J., et al. Blood 75:1311-1318 (1990)), utilizing exogenously provided purified IL-14, to overcome spontaneous apoptosis occurring in the initial weeks of cell culture.

EXAMPLE 16

Partial purification of IL-14 from effusion fluids.

Effusion fluids from NHL-B patients were immediately processed (within one hour) after the removal from the patient. After treatment with protease inhibitors, and passage through a 0.80μ and 0.22μ filters, the fluid was equilibrated through a PD-10 column (Pharmacia) into 50 mM Tris, pH 8.0, and then loaded onto a DEAE Sephacel column (Pharmacia) 20 ml bed volume, pre-equilibrated with 50 mM Tris pH 8.0. An NaCl gradient (1-500 mM) was run at 60 ml/hr. The individual fractions (2.5 ml) were collected and

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assayed at 10% v/v per well for growth factor activity (possibly autocrine or paracrine) on simultaneously obtained autochthonous patient NHL-B-cells and purified normal anti-μ-stimulated human peripheral blood B lymphocytes. A standard tritiated thymidine incorporation assay for assessing BCGF activity was utilized. In vitro growth factor activity for autochthonous NHL-B-cells was present in the chromatographically-fractionated effusion fluids. (Figure 5). Correspondingly, BCGF activity, determined on anti-μ stimulated peripheral blood B lymphocytes, was found in the same effusion fluid fractions of NHL-B patients (Figure 6, patients 1-4), but not in the control patients' fluids (Figure 6, controls a-c), suggesting that the same factor(s) or possibly related factors could be stimulating the lymphoma cells, and a putative normal B lymphocytic counterpart.

EXAMPLE 17

Immunologic identification of IL-14 (HMW-BCGF) in effusion fluids.

Effusions were fractionated by DEAE chromatography. Fractions with BCGF activity were collected and pooled, and subjected to Western blot analysis and immunoprecipitation with monospecific anti-HMW-BCGF polyclonal antibodies. Western blots were performed by standard techniques. Subsequently, 80μl of the peak fractions containing BCGF activity were separated on a 10% SDS-PAGE gel, electrophoresed and blotted onto nitrocellulose, blocked with blotto (3% powdered milk, 50mM Tris, 0.9% Tween, 0.02% NaN_3, pH 7.8) at 4°C overnight. Blots were then incubated with monospecific anti-HMW-BCGF (Goldstein, H., et al. Cell Immunol. 108:343-351 (1987)) polyclonal antibody (1:4000), for 2 hrs. at room temperature. The filters were then incubated with ^{125}I-labeled goat anti-rabbit Ig antisera (1:1000) (Amersham) for 1 hr. and exposed on x-ray film (Kodak XR). Imunoprecipitation was performed by incubating

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active peak DEAE fractions at 4°C with 40µg of purified anti-HMW-BCGF IgG or pre-immune IgG (negative control). The sample was then incubated with pre-equilibrated Protein A sepharose beads (50µl) (Sigma, St. Louis) for 1 hr. Beads and supernatant were separated using a millipore 0.22µ microfuge filter. The beads were extensively washed. At that time, 5x sample buffer was added to bound (beads) and unbound (supernatant), and fractions were run on 10% SDS-PAGE, transferred to nitrocellulose for analysis by Western blot analysis as above.

EXAMPLE 18

In vitro production of growth factor by NHL-B cells from effusion fluids.

To further verify that the growth factor activity present in the effusion fluids was produced by the NHL-B-cells present in these fluids, and not by a contaminating accessory cell (e.g. mesothelial cells), fresh NHL-B-cells were pelleted from effusion fluids, extensively washed, E-rosetted to remove contaminating T lymphocytes, and spun through a Ficoll/Hypaque (F/H; Sigma) gradient, washed, and cultured for 48 hrs in the presence or absence of PHA (0.75% v/v; GIBCO, Grand Is., NY) in RPMI, 10% FCS for 48 hrs. Monocytes/macrophages, and mesothelial cells (which were particularly scarce in these patients' fluids) were deleted by plastic adherence. NHL-B-cells accounted for >98% of the final lymphoma cell population by flow cytometric analysis. Control NHL-B-cells were cultured in FCS alone. Established cell lines from similar forms of NHL-B were treated similarly, and used as positive controls. Supernatants from cell cultures were clarified through a 0.22µ filter and concentrated in an Amicon concentrator using YM-10 membrane. Samples were then passed through a PD-10 column (Pharmacia, Piscataway, NJ) and subsequently tested for BCGF activity, as described below.

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Figure 7(a) shows the B-cell proliferative activity of NHL-B cell populations from patients. Patients whose fluids contained BCGF activity also had NHL-B-cells that produced BCGF activity in vitro. Similar results were obtained for established NHL-B-cell lines from other patients, including the Namalva cell line, from which HMW-BCGF was originally described (Figure 7(b)). Similar activities were observed when the autochthonous NHL-B-cells (as in Example 16) were used as target cells for the proliferation assay.

EXAMPLE 19

In vitro growth factor assays.

Growth factor assays for BCGF activity were performed as previously described (Maizel, A., et al. Proc. Natl. Acad. Sci. USA. 80:457-462 (1983)), utilizing anti-IgM- (anti-μ)-stimulated B lymphocytes obtained from normal donor peripheral blood mononuclear cell populations. B-cells were purified utilizing SRBC rosetting and separation on F/H, followed by plastic adherence to remove monocytes. Residual T cells or monocytes were removed, magnetically if necessary by further treatment with cocktails of MAb (e.g. CD2, 4, 14), followed by magnetic beads attached to anti-mouse Ig (Dynal, Great Neck, NY). B-cells utilized were greater than 95% CD 19 positive. Microtiter wells containing 1×10^5 anti-μ B-cells were exposed to growth factor containing fluid or supernatant fractions in a dose response fashion in triplicate. ^{3}H-thymidine uptake was determined over the last 18 hours of a 96 hr culture period. Freshly obtained patient NHL-B-cells were utilized in similar assays to assess response to exogenously provided growth factors, including HMW-BCGF (IL-14) purified from PHA-stimulated Namalva cell culture supernatants as previously described (Ambrus, J. L., et al. J. Clin. Invest. 75:732-739 (1985)), recombinant IL-2 (rIL-2; Cetus-Chiron, Emeryville, CA), or recombinant IL-4 (rIL-4; Genzyme, Cambridge, MA).

To determine the responsiveness of the freshly-obtained NHL-B-cells from the effusion fluids, to putative autocrine growth factor(s)/BCGFs produced by these lymphoma cells, we exposed the tumor cells to purified BCGFs in vitro. Two of the patients' NHL-B-cells responded actively to exogenously provided IL-14 (HMW-BCGF), but not to other putative B-cell growth promoting cytokines, such as recombinant IL-2 or IL-4 (rIL-2, rIL-4). Another of the patients had a very high spontaneous proliferation, that was not significantly increased with the addition of exogenous HMW-BCGF or other putative B-cell stimulatory cytokines, but was inhibited by rIL-4.

EXAMPLE 20

Specific antibody blocking of HMW-BCGF (IL-14) activity in effusion fluids and in vitro culture supernatants.

Partially purified HMW-BCGF activity (pooled DEAE active fractions) from freshly obtained effusion fluids were concentrated 10x through a 10,000 MW cutoff membrane (Amicon Centriprep 10) and desalted into RPMI media with a PD 10 column (Pharmacia). Conditioned media (CM) from cultures of freshly obtained NHL-B-cells were concentrated 10x in a similar fashion. Anti-HMW-BCGF polyclonal IgG antibody (4 mg) or preimmune control rabbit IgG was bound to 500 µl bed volume of RPMI-equilibrated Protein A Sepharose beads (Sigma). 500 ml of the concentrated active fractions (effusion fluids or CMs) were incubated with the antibody bound or control Ig-bound beads for 12 hours at 4°C. The supernatants were then passed through microfuge filter units (Millipore Ultrafree MC, 0.45µ), and immediately assayed for growth factor activity.

If autocrine growth factor activity mediated by HMW-BCGF (IL-14), is responsible for lymphoma cell growth, it should be possible to block growth stimulation with specific antibodies. To address this point, the freshly obtained

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effusion fluids and conditioned media obtained after in vitro culture of purified NHL-B-cells were treated with anti-HMW-BCGF antibody attached to sepharose beads. When effusion fluids and conditioned media were treated with antibody bound beads for 12 hours at 4°C, >70% of the growth factor activity was removed in both instances. Effusion fluids and CM treated with control (non-immune) rabbit IgG-conjugated beads had <5% of the growth factor activity removed.

EXAMPLE 21

HMW-BCGF (IL-14) gene expression analysis utilizing polymerase chain reaction (PCR).

Since we were able to confirm that the cytokine growth factor present in the patients' effusions had the biologic and immunologic characteristics of IL-14, we examined IL-14 mRNA.

Freshly obtained NHL-B-cells from effusion fluids were processed as described above, and total or polyA RNA was extracted utilizing standard techniques. Oligonucleotide primers based on the HMW-BCGF cDNA sequence disclosed herein (See also, Ambrus, J. L., et al. Proc. Natl. Acad. Sci. (USA) 90:6330-6334 (1993)). Reverse transcriptase (RT)-PCR analysis was performed using a Thermo Cycler 480 (Perkin-Elmer-Cetus) utilizing Taq polymerase, and 35 cycles of amplification. The amplified material was then stained with ethidium bromide, run on a 1.2% agarose gel, and photographed under UV illumination. Amplified bands were confirmed by Southern blot analysis using a radioactively labeled IL-14 cDNA probe (Ambrus, J. L., et al. Proc. Natl. Acad. Sci. (USA) 90:6330-6334 (1993)).

The IL-14 mRNA (1.3 Kb band), was identified in each of the patients' NHL-B-cells. Controls included the Namalva Burkitt's lymphoma cell line (which secretes HMW-BCGF) and normal peripheral blood monocytes (which do not secrete

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HMW-BCGF). Southern blot analysis confirmed these findings, using a cDNA probe for IL-14.

Those skilled in the art will recognize or will be able to ascertain with no more than routine experimentation numerous equivalents to the specific products and processes described herein. Such equivalents are considered to be within the scope of the invention and are intended to be covered by the following claims in which we claim:

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: WASHINGTON UNIVERSITY, ST. LOUIS
(B) STREET: 1 BROOKINGS DRIVE
(C) CITY: ST. LOUIS
(D) STATE: MISSOURI
(E) COUNTRY: UNITED STATES
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(E) COUNTRY: UNITED STATES
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(G) TELEPHONE: 713-529-4045

(ii) TITLE OF INVENTION: HIGH MOLECULAR WEIGHT B-CELL GROWTH FACTOR: INTERLEUKIN-14

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: WOLF, GREENFIELD & SACKS, P.C.
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(C) CITY: BOSTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: USA
(F) ZIP: 02210

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: NOT AVAILABLE
(B) FILING DATE: FILED HEREWITH

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/005,156
(B) FILING DATE: 15-JAN-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: GATES, EDWARD R.
(B) REGISTRATION NUMBER: 31,616
(C) REFERENCE/DOCKET NUMBER: B0819/7000WO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/720-3500
(B) TELEFAX: 617/720-2441

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1854 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

BNSDOCID: <WO__9416074A2_I_>

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(G) CELL TYPE: LYMPHOMA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AAGGGCAGGG GAAGCGATGC CAAGGGGCTC CTGACCAGCA CATCATCCCA CGCAAAAACA 180

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CAGACAGAGA AATGCTCAAG TCACTTCTGC CAACTCACTG TGATGGCAGC TACAGATGAC 480

AGCCCCTCTC AAGACTCTTC AGCTCACAGA CAAGCCACTG ACTTCATCTG TACACACCCC 540

CATCCCCAAT GCAAGCTCCA CTGTACACTT ACAGGTATAA ATGCATTTGC AAGGCCTTGC 600

AAAATGCCCT ATGTACGTAA AACTGACCCA CAAAAGTGCC AAAATTGCAA GTGCCAGATG 660

CCAGCCAGGT CAGAACATGC CTGGCTTCAG CAATGGGCTG CTCAGCATGG GAGCCTTTTA 720

TGGGCCAGGC TGGCTGGGCT GCCGCTCCCT TCCCAGCATG ACCCAACACC AGGCTCTCTA 780

GGCCCTGGCG GAGGTGGGCT CTTGAGGCCA GTCTGGCCTG ATGCTTCTGT GCTCGGTTGC 840

TCCTGGGTAG CAAGGCGCTT CTGTGACCCT GGGGGAGCTG GGTGCTTGAT GCCCCAGTGC 900

CCCTCTGGCC TCCTCTCAGG GCCACTGTCA GTGAGGGAGC CCTGGCCACC AGCACTCAGG 960

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BNSDOCID: <WO 9416074A2 | >

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AAGACTTTGT CGATATGCTC CTCGCGCAGC TCATACTGCT CAATCAGCTT CTTGAGCCTC 1500

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GGTGGTCCTT CTCTTGCACC AGCTGGCTCT GCTTTTTCTG TAGGAGCTTC ATCTGCTTCA 1800

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 498 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 16..498

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ile Arg Leu Leu Arg Ser Ile Ile Gly Leu Val Leu Ala Leu Pro
-15                 -10                 -5                  1

Lys Gly Arg Gly Ser Asp Ala Lys Gly Leu Leu Thr Ser Thr Ser Ser
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His Ala Lys Thr Phe Ser Arg Ser Leu Val Ser Arg Gln Glu Ile Pro
        20                  25                  30

Ser Ser Glu Arg Pro Ala Arg Leu Cys Leu Gly Thr Pro Phe Leu Arg
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BNSDOCID: <WO___9416074A2_I_>

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Leu Asp Leu Ser Lys Pro Cys Ser Ser Arg Gln Met Gln Phe Asn Asp
70 75 80

Leu Gly Val Leu Leu Val Leu Ser Ser Glu Gly Gln Gly Gly Val Ile
85 90 95

Glu Pro Gln Ser Gln Thr Glu Lys Cys Ser Ser His Phe Cys Gln Leu
100 105 110

Thr Val Met Ala Ala Thr Asp Asp Ser Pro Ser Gln Asp Ser Ser Ala
115 120 125

His Arg Gln Ala Thr Asp Phe Ile Cys Thr His Pro His Pro Gln Cys
130 135 140 145

Lys Leu His Cys Thr Leu Thr Gly Ile Asn Ala Phe Ala Arg Pro Cys
150 155 160

Lys Met Pro Tyr Val Arg Lys Thr Asp Pro Gln Lys Cys Gln Asn Cys
165 170 175

Lys Cys Gln Met Pro Ala Arg Ser Glu His Ala Trp Leu Gln Gln Trp
180 185 190

Ala Ala Gln His Gly Ser Leu Leu Trp Ala Arg Leu Ala Gly Leu Pro
195 200 205

Leu Pro Ser Gln His Asp Pro Thr Pro Gly Ser Leu Gly Pro Gly Gly
210 215 220 225

Gly Gly Leu Leu Arg Pro Val Trp Pro Asp Ala Ser Val Leu Gly Cys
230 235 240

Ser Trp Val Ala Arg Arg Phe Cys Asp Pro Gly Gly Ala Gly Cys Leu
245 250 255

Met Pro Gln Cys Pro Ser Gly Leu Leu Ser Gly Pro Leu Ser Val Arg
260 265 270

Glu Pro Trp Pro Pro Ala Leu Arg Ser Cys Thr Leu Leu Phe Arg Ser
275 280 285

Leu Arg Ser Val Cys Ser Ala Arg His Ser Phe Ser Ser Arg Trp Ile
290 295 300 305

Phe Thr Cys Arg Pro Ser Ser Ser Leu Ser Arg Thr Val Phe Ser Ser
310 315 320

Ala Ile Ser Ser Arg Ala Leu Leu Leu Leu Ser His Arg Asp Arg Tyr
325 330 335

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Met Val Val Ser Phe Ser Ser Phe Leu Ile Phe Leu Val Ile Phe Ser
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Ile Ser Cys Leu Asn Val Val Asn Thr Ser Leu Leu Leu Glu Ser Val
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Phe Trp Asn Ser Ser Asn Phe Ser Val Tyr Arg Ala Ser Cys Cys Phe
370                 375                 380                 385

Arg Trp Val Ser Cys Cys Phe Ile Ser Ser His Ile Leu Trp Asp Ser
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Thr Ala Ser Phe Arg Arg Lys Ser Phe Ser Arg Trp Cys Arg Ser Ser
            405                 410                 415

Ala Ser Phe Ser Ile Ser Trp Ala Cys Trp Ser Leu Ala Ser Thr Ser
        420                 425                 430

Cys Cys Cys Arg Ser Leu Cys Leu Lys Thr Leu Ser Ile Cys Ser Ser
    435                 440                 445

Arg Ser Ser Tyr Cys Ser Ile Ser Phe Leu Ser Leu Ser Ala Ser Ser
450                 455                 460                 465

Met Phe Ser Trp Arg Ser Leu Glu Leu Arg Ser Leu Cys Cys Ser Ile
                470                 475                 480

Cys Ser
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Gln Phe Asn Asp Leu Gly Val Leu Leu
1               5
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(2) INFORMATION FOR SEQ ID NO:4:

BNSDOCID: <WO___9416074A2_I_>

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Ala Thr Asp Asp Ser Pro Ser
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Pro Gln Cys Pro Ser Gly Leu Leu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Pro Lys Gly Arg Gly Ser Asp Ala Lys Gly Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Ser Leu Val
1

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Tyr Val Arg Lys Thr Asp Pro Gln Lys
1 5 10

BNSDOCID: <WO___9416074A2_I_>

CLAIMS

1. An isolated nucleic acid encoding at least a fragment of a high molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation.

2. An isolated nucleic acid as in claim 1 wherein said growth factor is an Interleukin-14.

3. An isolated nucleic acid as in claim 1 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

4. An isolated nucleic acid as in claim 2 or 3 wherein said nucleic acid comprises at least of fragment of SEQ.ID.NO.:1.

5. An isolated nucleic acid as in claim 2 or 3 wherein said growth factor comprises at least a fragment of SEQ.ID.NO.:2.

6. An isolated nucleic acid comprising a sequence which is a substantially similar variant of at least a fragment of SEQ.ID.NO.:1.

7. An isolated nucleic acid as in claim 1 wherein the nucleic acid encodes the entire open reading frame of a high molecular weight B-cell growth factor.

8. An isolated nucleic acid as in claim 7 wherein the nucleic acid encodes a human high molecular weight B-cell growth factor.

9. An isolated nucleic acid as claimed in claim 1 wherein the nucleic acid is SEQ.ID.NO.:1.

10. A substantially pure protein comprising at least a fragment of a high molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation.

11. A substantially pure protein as in claim 10 wherein said growth factor is an Interleukin-14.

12. A substantially pure protein as in claim 10 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

13. A substantially pure protein as in claim 11 or 12 wherein said protein comprises at least a fragment of SEQ.ID.NO.:2.

14. A substantially pure protein comprising a polypeptide sequence which is a substantially similar variant of at least a fragment of SEQ.ID.NO.:2.

15. A substantially pure protein as in claim 10, wherein said protein is a high molecular weight B-cell growth factor.

16. A substantially pure protein as in claim 15, wherein said protein is an interleukin-14.

17. A substantially pure protein as in claim 16, wherein said protein is human interleukin-14 having the sequence of SEQ.ID.NO.:2.

18. A substantially pure protein comprising a fusion of a cytotoxic polypeptide and at least a fragment of a high

molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation.

19. A substantially pure protein as in claim 18 wherein said growth factor is an Interleukin-14.

20. A substantially pure protein as in claim 18 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

21. A substantially pure protein as in claim 19 or 20 wherein said fragment comprises at least a fragment of SEQ.ID.NO.:2.

22. A method of producing at least a fragment of a high molecular weight B-cell growth factor comprising:

introducing an expression vector into a host, said expression vector including a nucleic acid sequence encoding a high molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation, wherein said nucleic acid sequence is operably joined to regulatory sequences such that said growth factor is produced;

allowing said host to produce said growth factor; and

substantially purifying said growth factor.

23. A method as in claim 22 wherein said nucleic acid sequence is at least a fragment of SEQ.ID.NO.:1.

24. A method as in claim 22 wherein said nucleic acid sequence encodes at least a fragment of SEQ.ID.NO.:2.

25. A substantially pure antibody capable of selectively binding at least a fragment of a high molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation.

26. A substantially pure antibody as in claim 25 wherein said growth factor is an Interleukin-14.

27. A substantially pure antibody as in claim 25 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

28. A substantially pure antibody as in claim 26 or 27 wherein said growth factor comprises at least a fragment of SEQ.ID.NO.:2.

29. A substantially pure antibody as in any one of claims 25 to 28 wherein said antibody is monoclonal.

30. A cytotoxic composition comprising a toxin moiety coupled to at least a fragment of a high molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation.

31. A cytotoxic composition as in claim 30 wherein said growth factor is an Interleukin-14.

32. A cytotoxic composition as in claim 30 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

33. A cytotoxic composition as in claim 31 or 32 wherein said growth factor comprises at least a fragment of SEQ.ID.NO.:2.

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34. A cytotoxic composition comprising a toxin moiety coupled to a polypeptide comprising a substantially similar variant of at least a fragment of SEQ.ID.NO.:2.

35. A cytotoxic composition as in any one of claims 30 to 33, wherein said toxin moiety is selected from the group consisting of a high-energy emitting radionuclide, abrin, gelonin, ricin, Pseudomonas exotoxin, diptheria exotoxin, and pokeweed antiviral protein.

36. An isolated nucleic acid comprising a nucleotide sequence encoding an RNA transcript which is anti-sense to at least a fragment of an mRNA transcript of a high molecular weight B-cell growth factor wherein said growth factor stimulates B-cell proliferation and inhibits B-cell differentiation.

37. An isolated nucleic acid as in claim 36 wherein said growth factor is an Interleukin-14.

38. An isolated nucleic acid as in claim 36 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

39. An isolated nucleic acid as in claim 37 or 38 wherein said nucleic acid comprises a nucleotide sequence complementary to at least a fragment of SEQ.ID.NO.:1.

40. An isolated nucleic acid comprising a nucleotide sequence which is complementary to a substantially similar variant of at least a fragment of SEQ.ID.NO.:1.

41. An isolated nucleic acid comprising an RNA transcript which is anti-sense to at least a fragment of an

BNSDOCID: <WO 9416074A2 | >

mRNA transcript of a high molecular weight B-cell growth factor wherein said growth factor is capable of stimulating B-cell proliferation and inhibiting B-cell differentiation.

42. An isolated nucleic acid as in claim 41 wherein said growth factor is an Interleukin-14.

43. An isolated nucleic acid as in claim 41 wherein said growth factor is a mammalian high molecular weight B-cell growth factor having an isoelectric point of approximately 7.8.

44. An isolated nucleic acid as in claim 42 or 43 wherein said nucleic acid comprises at least a fragment of SEQ.ID.NO.:1.

45. An isolated nucleic acid comprising an RNA transcript which is a substantially similar variant of at least a fragment of SEQ.ID.NO.:1.

46. A composition useful for inhibiting B-cell proliferation in vivo comprising the antibody of claim 25 and a pharmaceutically acceptable carrier.

47. A composition useful for inhibiting B-cell proliferation in vivo comprising the protein of claim 18 and a pharmaceutically acceptable carrier.

48. A composition useful for inhibiting B-cell proliferation in vivo comprising the cytotoxic composition of claim 30 and a pharmaceutically acceptable carrier.

49. A composition useful for inhibiting B-cell proliferation in vivo comprising the nucleic acid of claim 36 operably joined to regulatory sequences in an expression vector that expresses the nucleic acid in mammalian B-cells.

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50. A composition useful for stimulating B-cell proliferation in vivo comprising the protein of claim 10 and a pharmaceutically acceptable carrier.

51. A composition as in claim 50 further comprising an immunogen, and wherein the protein and the immunogen are formulated as a vaccine.

52. A composition as in claim 51 further comprising at least one additional immunostimulatory cytokine.

53. A composition as in claim 50 further comprising lymphocytes.

54. A method for stimulating B-cell proliferation comprising exposing a culture of B-cells to the protein of claim 10.

55. A method of selectively depleting a population of cells having receptors for a high molecular weight B-cell growth factor, comprising contacting the cells with a cytotoxin that selectively binds the cells, the cytotoxin including the protein of claim 10 and selectively binding to cells via that protein.

56. A method of selectively interfering with the replication of B-cells, comprising contacting said B-cells with an anti-high molecular weight B-cell growth factor agent.

57. A method as in claim 56 wherein said B-cells are cancer cells.

58. A method as in claim 56 wherein said B-cells are producing autoimmune antibodies.

59. A method as in claim 56 wherein said B-cells are Interleukin-14 dependent and said agent is selected from the group consisting of immunotoxins and anti-sense.

60. A method for enhancing the immune system of a patient comprising administering to the patient a high molecular weight B-cell growth factor.

61. A method for enhancing the immune system of a patient comprising administering to the patient autologous B-cells that have been proliferated in vitro using a high molecular weight B-cell growth factor.

62. A recombinant cell line comprising a host cell including the nucleic acid of claim 1 operably joined to regulatory sequences.

63. A transgenic animal including the nucleic acid of claim 1 operably joined to regulatory sequences.

64. A recombinant cell line comprising a host cell in which a high molecular weight B-cell growth factor gene has been inactivated by homologous recombination using at least a fragment of SEQ.ID.NO.:1.

65. A transgenic animal in which a high molecular weight B-cell growth factor gene has been inactivated by homologous recombination using at least a fragment of SEQ.ID.NO.:1.

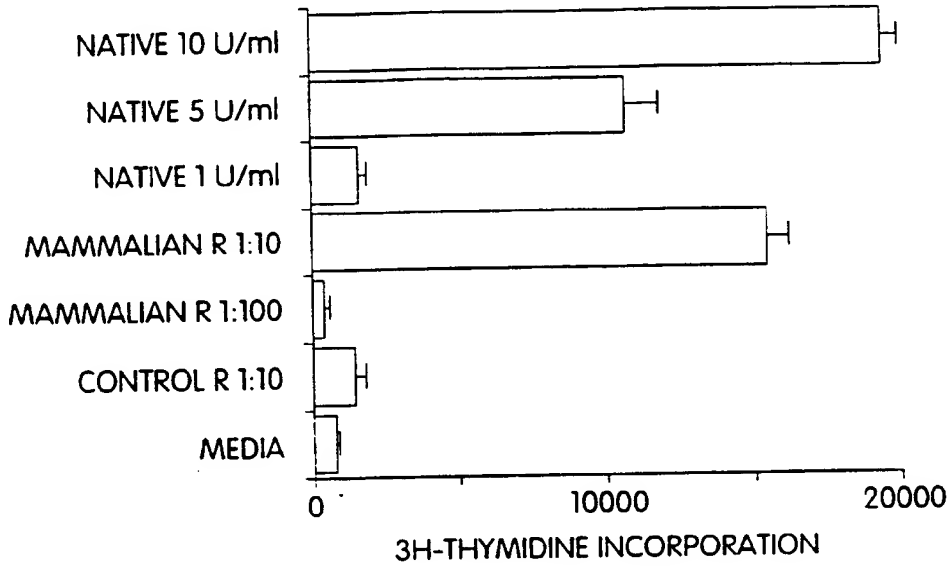
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Fig. 1

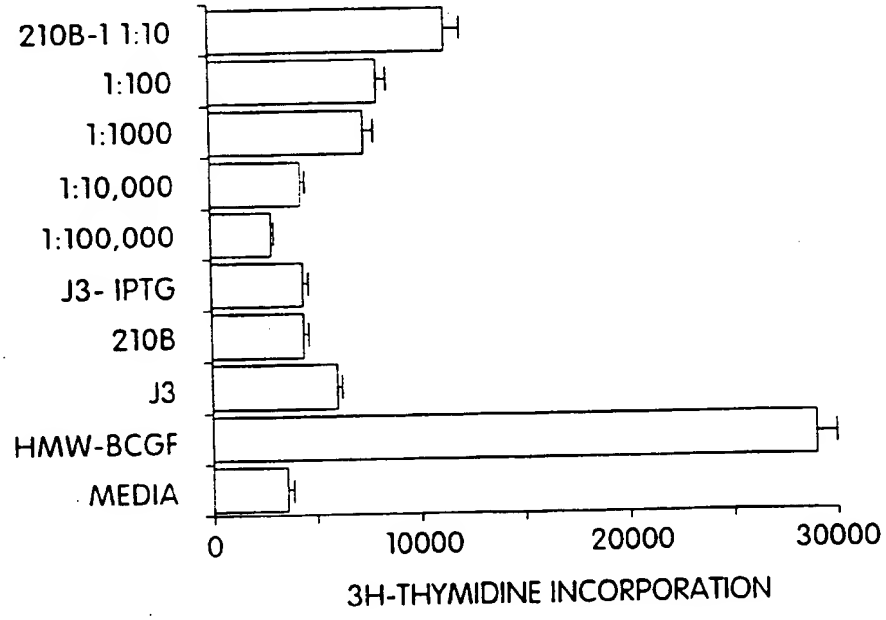
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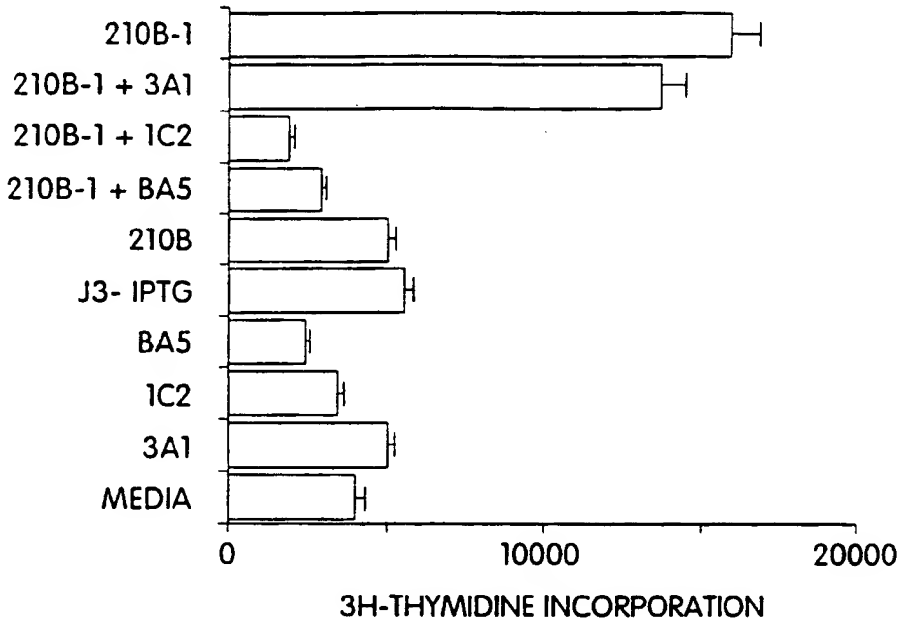
Fig. 2



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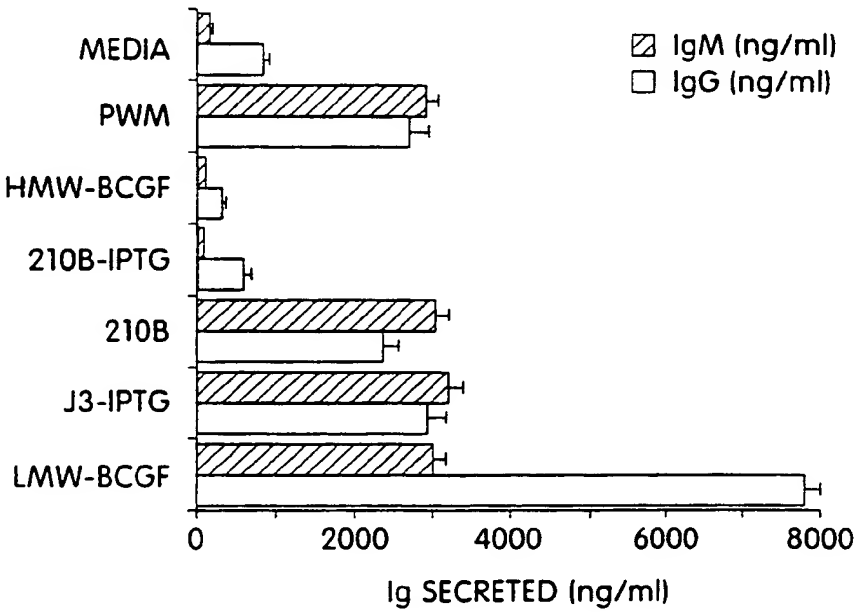

Fig. 4

BNSDOCID: <WO 9416074A2 1 >

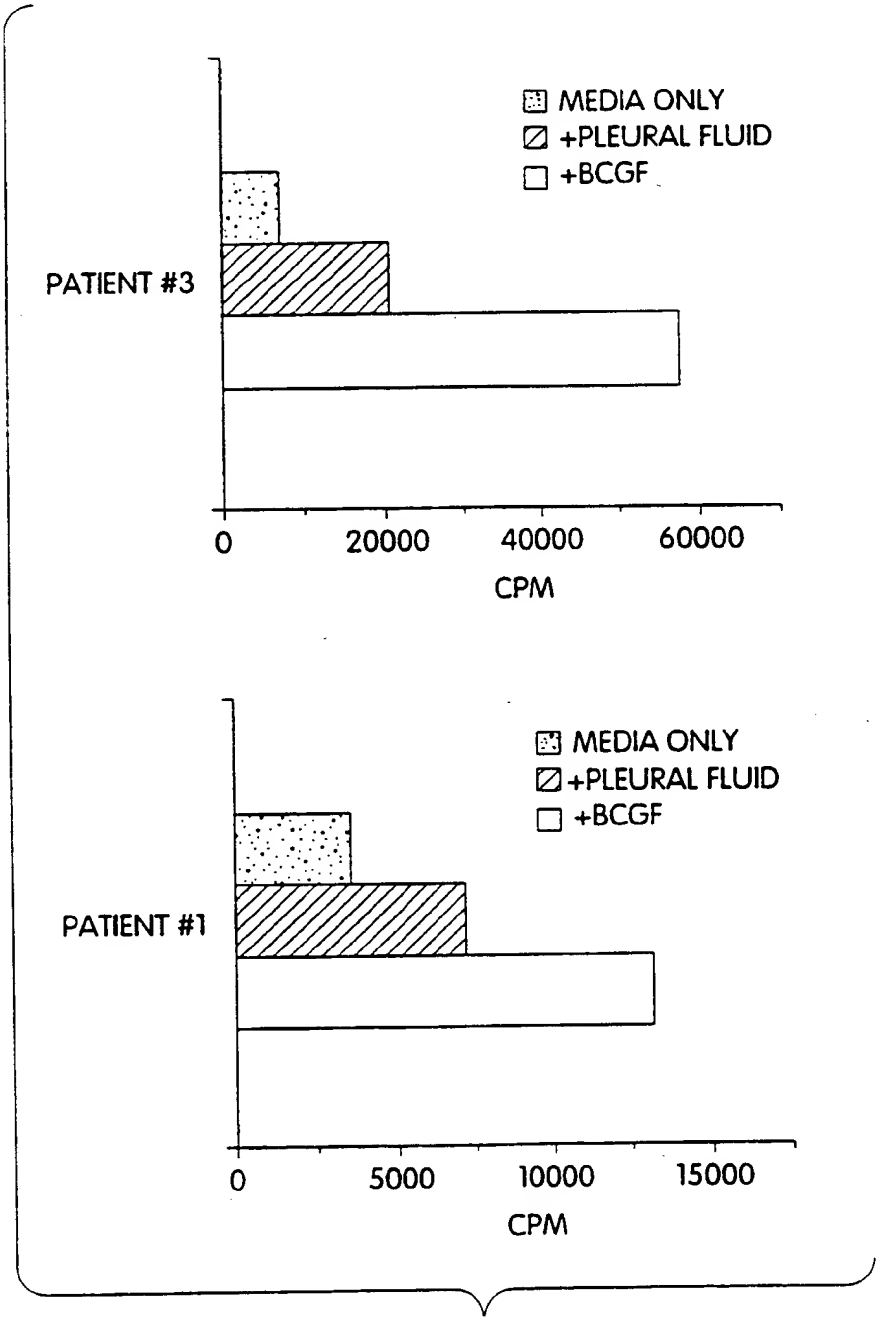
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Fig. 5

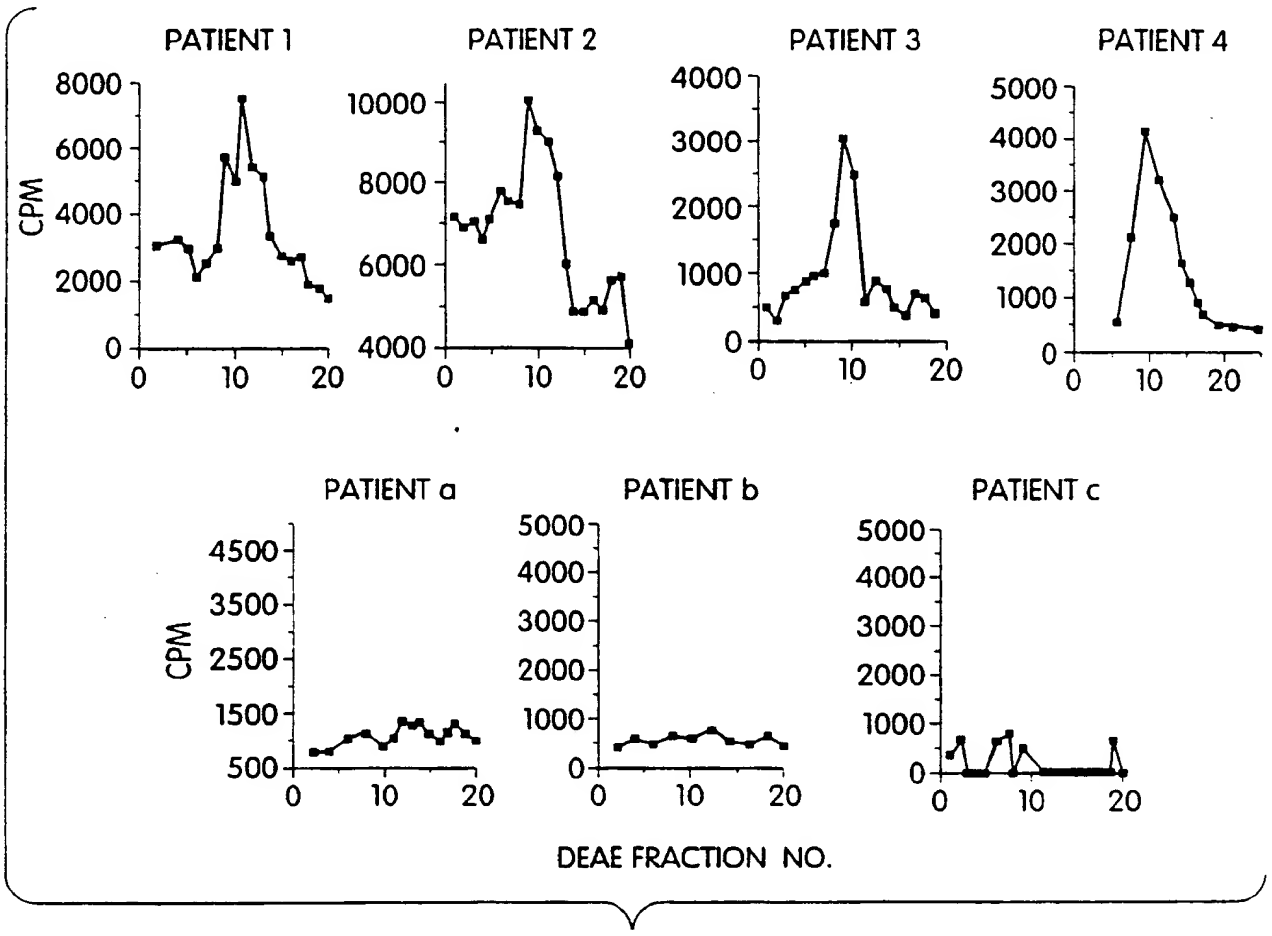

Fig. 6

BNSDOCID: <WO__ 9416074A2 I >

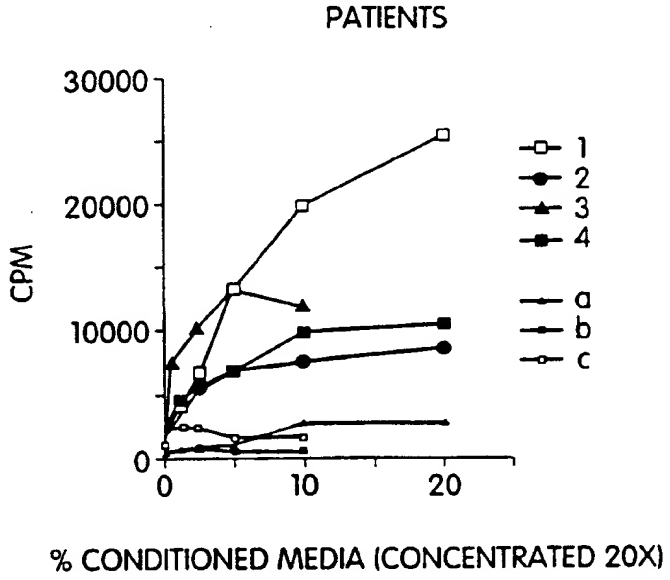

Fig. 7a

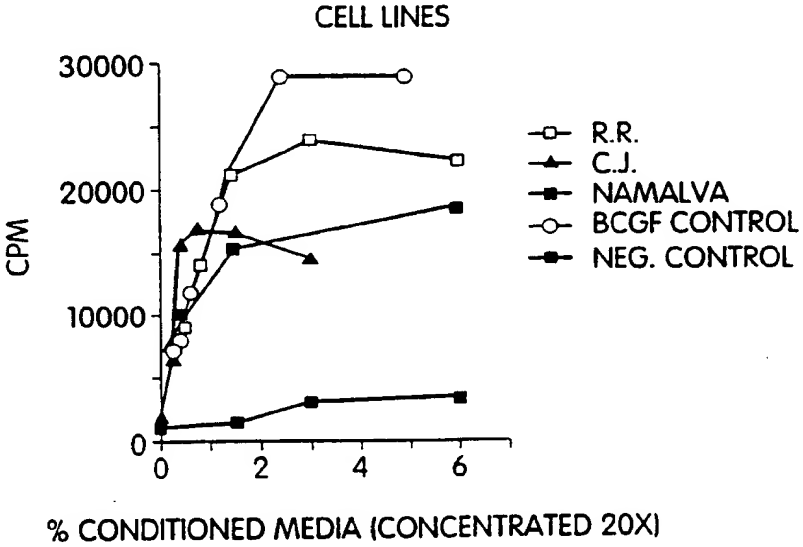

Fig. 7b

BNSDOCID: <WO 9416074A2 I >

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/24, C12P 21/02, C07K 13/00, A61K 37/02, C12N 15/62, 5/10, C12P 21/08, C12N 15/11, A61K 31/70, 35/14 A61K 39/395, A01K 67/027, C12N 15/63

A3

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(54) Title: HIGH MOLECULAR WEIGHT B-CELL GROWTH FACTOR: INTERLEUKIN-14

(57) Abstract

A mammalian high molecular weight B-cell growth factor nucleotide has been cloned and sequenced. Recombinant vectors and cells are described. Methods of providing isolated high molecular weight B-cell growth factor DNA and polypeptide sequences are disclosed, as well as methods of making transgenic animals containing or lacking the high molecular weight B-cell growth factor sequence. Clinical uses of HMW-BCGF and conjugates thereof are also described.

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International Application No
PCT/US 94/01101

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/24 C12P21/02 C07K13/00 A61K37/02 C12N15/62
C12N5/10 C12P21/08 C12N15/11 A61K31/70 A61K35/14
A61K39/395 A01K67/027 C12N15/63

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol.162, no.4, 1 October 1985 pages 1319 - 1335 J. L. AMBRUS ET AL 'Purification to homogeneity of a high molecular weight human B cell growth factor ; demonstration of specific binding to activated B cells ; and development of a monoclonal antibody to the factor' cited in the application see the whole document --- -/--	1-17

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1994	10. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Le Cornec, N

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol.90, July 1993, WASHINGTON US pages 6330 - 6334 J. L. AMBRUS ET AL 'Identification of a cDNA for a high- molecular-weight B-cell growth factor' cited in the application see the whole document	1-17, 22-24, 40,45, 54,62
X	JOURNAL OF IMMUNOLOGY, vol.145, no.12, 15 December 1990, BALTIMORE US pages 3949 - 3955 J. L. AMBRUS ET AL 'Functional studies examining the subpopulation of human B lymphocytes responding to high molecular weight B cell growth factor' cited in the application see the whole document	1-17,54
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol.266, no.6, 25 February 1991, BALTIMORE, MD US pages 3702 - 3708 J.L. AMBRUS ET AL 'Intracellular signaling events associated with the induction of proliferation of normal human B lymphocytes by two different antigenically related human B cell growth factors (high molecular weight B cell growth factor (HMW-BCGF) and the complement factor Bb' see the whole document	1-17,54
X	THE JOURNAL OF CLINICAL INVESTIGATION, vol.75, no.2, February 1985 pages 732 - 739 J.L. AMBRUS ET AL 'human B lymphoma cell line producing B cell growth factor' cited in the application see the whole document	1-17,54
X	ALLERGY AND CLINICAL IMMUNOLOGY, vol.87, no.6, June 1991 pages 1138 - 1149 J.L. AMBRUS ET AL 'Abnormal response to a human B cell growth factor in patients with common variable immunodeficiency (CVI)' cited in the application see the whole document	1-17,54

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INTERNATIONAL SEARCH REPORT

I national application No.
PCT/US94/01101

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see annex

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-17,22-24,36-44,45,
49,54,61,(62-65)partially 56-59

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1.- Claims: 1-17, 22-24, 36-44, 45, 49, 62-65 and partially 56-59

Nucleic acids encoding HMW-BCGF, the HMV-BCGF protein method to produce it by r DNA technology, host cells transformed to express it and transgenic animal expressing it. A recombinant cell line, a transgenic animal in which HMW-BCGF gene has been inactivated by homologous recombination. Antisense to a.m. RNA transcript of a HMW-BCGF. Composition to inhibit B-cell proliferation using an antisense in an expression vector that expresses it in mammalian cells. Method for selectively interfering with the replication of B-cells using the anti-sense.

2.- Claims: 18-21, 30-35, 47-48, 55

A fusion of a cytotoxic polypeptide and a HMW-BCGF, its use in a pharmaceutical composition, a cytotoxic composition comprising a toxin moiety coupled to a HMW-BCGF and its use in a pharmaceutical composition.

3.- Claims: 25-29, 46

Antibodies to HMW-BCGF and their use in a pharmaceutical composition. Method to selectively interfering with the replication of B-cells using them.

4.- Claims 50-53, 60

Pharmaceutical composition containing HMW-BCGF, method to enhance the immune system using a HMW-BCGF.

5.- Claims: 54, 61

Method for stimulating B-cell proliferation comprising exposing a culture of B-cells to the protein HMW-BCGF and method to enhance the immune system of a patient comprising administering these B-cells.

6.- Claims: 56-59 partially

Method to selectively interfering with the replication of B-cells using an anti-high molecular weight B-cell growth factor agent: immunotoxins.

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